# Texas Nonpoint Source Grant Program

## *Bacteria Growth, Persistence, and Source Assessment in Rural Texas Landscapes and Streams*

***TSSWCB Project 13-56***

***Revision #0***

# Quality Assurance Project Plan

# Texas State Soil and Water Conservation Board

Prepared by:


Texas Water Resources Institute

Texas A&M AgriLife Research – Department of Biological and Agricultural Engineering

Texas A&M AgriLife Research Soil and Aquatic Microbiology Lab

Texas A&M Institute of Renewable Natural Resources

United States Department of Agriculture – Agricultural Research Service

Texas A&M AgriLife Research Nutrient and Water Analysis Lab


Effective Period: Upon TSSWCB Approval through May 31, 2015

Questions concerning this quality assurance project plan should be directed to:


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# Section A1: Approval Sheet

Quality Assurance Project Plan (QAPP) for the *Bacteria Growth, Persistence, and Source Assessment in Rural Texas Landscapes and Streams.*

**Texas State Soil and Water Conservation Board (TSSWCB)**

Name: Wesley Gibson
Title: TSSWCB Project Manager (PM)

**Signature:_____ Date: _____**

Name: Pamela Casebolt
Title: TSSWCB Quality Assurance Officer (QAO)

**Signature:_____ Date: _____**

**Texas A&M AgriLife Research – Texas Water Resources Institute (TWRI)**

Name: Kevin Wagner, PhD
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Name: Lucas Gregory
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Name: R. Karthikeyan, PhD
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**Signature:_____ Date: _____**

Name: Saqib Mukhtar, PhD
Title: Professor, Interim Associate Department Head, Extension Program Leader & Project Co-Lead

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**Texas A&M AgriLife Research - Soil and Aquatic Microbiology Lab (SAML)**

Name: Terry Gentry, PhD
Title: Assoc. Prof. of Soil & Aquatic Microbiology; SAML Director & Project Co-Lead

**Signature:_____Date: _____**

**Texas A&M Institute of Renewable Natural Resources (IRNR)**

Name: Roel Lopez, PhD
Title: IRNR Director; IRNR Project Co-Lead

**Signature:_____Date:_____**

**United States Department of Agriculture – Agricultural Research Service, Grasslands Soil and Water Research Lab (USDA-ARS)**

Name: Daren Harmel, PhD
Title: Agricultural Engineer and Project Co-Lead

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**Texas A&M AgriLife Research – Nutrient and Water Analysis Lab (NAWA)**

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Title: Assistant Professor of Urban Nutrient and Water Management & Project Co-Lead

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# Section A2: Table of Contents

# List of Acronyms and Abbreviations

| | |
|---|---|
| AWRL | ambient water reporting limits |
| BAEN | Department of Biological and Agricultural Engineering |
| BST | Bacterial source tracking |
| CAR | corrective action report |
| CFU | colony forming unit |
| COC | chain of custody |
| DI | deionized |
| DO | dissolved oxygen |
| DOC | dissolved organic carbon |
| DQO | data quality objectives |
| DNA | Deoxyribonucleic acid |
| ERIC-PCR | Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction |
| ERIC-RP | ERIC-PCR and RiboPrinting combination method |
| *E. coli* | *Escherichia coli* |
| HDPE | high-density polyethylene |
| IRNR | Texas A&M Institute of Renewable Natural Resources |
| LCS | laboratory control sample |
| LCSD | laboratory control sample duplicate |
| LOQ | limit of quantitation |
| LU/LC | landuse/landocver |
| mTEC | membrane thermotolerant *E. coli* |
| MUG | 4-methylumbelliferyl-β-D-glucuronide |
| NA-MUG | nutrient agar with MUG |
| NAWA | Nutrient and Water Analysis Lab |
| NELAP | National Environmental Laboratory Accreditation Program |
| NIST | National Institute of Standards and Technology |
| PCR | polymerase chain reaction |
| PM | Project Manager |
| QA | quality assurance |
| QAPP | quality assurance project plan |
| QAO | Quality Assurance Officer |
| QC | quality control |
| QPR | quarterly progress report |
| RPD | Relative percent deviation |
| SAML | Texas A&M AgriLife Research- Soil and Aquatic Microbiology Lab |
| SOP | Standard operating procedure |
| TMDL | total maximum daily load |
| TSSWCB | Texas State Soil and Water Conservation Board |
| TWRI | Texas A&M AgriLife Research, Texas Water Resources Institute |
| USDA-ARS | United States Department of Agriculture-Agriculture Research Service |
| USEPA | United States Environmental Protection Agency |
| UV | ultraviolet |

# Section A3: Distribution List

Organizations, and individuals within, which will receive copies of the approved QAPP and any subsequent revisions include:

**Texas State Soil and Water Conservation Board**
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Title: TSSWCB PM

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Title: TSSWCB QAO

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**USDA – ARS, Grasslands Soil and Water Research Lab**
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Name: Daren Harmel, PhD
Title: Agricultural Engineer & Project Co-Lead

**Texas A&M AgriLife Research – Nutrient and Water Analysis Lab**
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Name: Jacqueline Aitkenhead-Peterson, PhD
Title: Assistant Professor of Urban Nutrient and Water Management & Project Co-Lead

# Section A4: Project/Task Organization

The following is a list of individuals and organizations participating in the project with their specific roles and responsibilities:

**TSSWCB** – Texas State Soil and Water Conservation Board, Temple, Texas. Provides project overview at the State level.

Wesley Gibson, TSSWCB PM

Responsible for ensuring that the project delivers data of known quality, quantity, and type on schedule to achieve project objectives. Tracks and reviews deliverables to ensure that tasks in the work plan are completed as specified. Reviews and approves QAPP and any amendments or revisions and ensures distribution of approved/revised QAPPs to TSSWCB participants.

Pamela Casebolt; TSSWCB QAO

Reviews and approves QAPP and any amendments or revisions. Responsible for verifying that the QAPP is followed by project participants. Monitors implementation of corrective actions. Coordinates or conducts audits of field and laboratory systems and procedures. Determines that the project meets the requirements for planning, quality assurance (QA), quality control (QC), and reporting under the Texas Nonpoint Source Program.

**TWRI** – Texas Water Resources Institute, College Station, Texas. Responsible for project coordination and administration; QAPP development; project reporting; website development; education and outreach; simulated instream *E. coli* response to water quality modifications; LU/LC source assessments.

Kevin Wagner, TWRI Associate Director; Project Co-Lead

The TWRI Project Lead is responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract and in the project QAPP; assessing the quality of subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB PM. Responsible for supporting the development and ensuring the timely delivery of project deliverables, ensuring cooperation between project partners, providing fiscal oversight and completing project reporting.

Lucas Gregory, TWRI Project Specialist, QAO; Project Co-Lead

Responsible for determining that the QAPP meets the requirements for planning, QA and QC. Conducts audits of field and laboratory systems and procedures. Responsible for maintaining the official, approved QAPP, as well as conducting quality assurance audits in conjunction with TSSWCB personnel. Responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract and in the project QAPP; assessing the quality of

subcontractor/participant work; and submitting accurate and timely deliverables to the TSSWCB PM. Responsible for supporting the development and ensuring the timely delivery of project deliverables, ensuring cooperation between project partners, providing fiscal oversight and completing project reporting.

**BAEN** – Texas A&M AgriLife Research – Department of Biological and Agricultural Engineering, College Station, Texas. Responsible for assessing simulated instream *E. coli* response to water quality modifications; LU/LC source assessments; education and outreach.

R. Karthikeyan, Associate Professor & Project Co-Lead

Responsible for overseeing simulated instream trials and landuse/landcover (LU/LC) source assessments. This includes ensuring that laboratory personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and the analyses or task performed. Responsible for oversight of all laboratory operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective action is implemented, documented, reported and verified. Monitors implementation of the measures within the laboratory to ensure complete compliance with project data quality objectives (DQOs) in the QAPP. Conducts in-house audits to ensure QAPP compliance and identify potential problems.

Saqib Mukhtar, Professor, Interim Associate Department Head & Project Co-Lead

Responsible for providing support performing simulated instream trials and LU/LC source assessments. Responsible for ensuring that tasks and other requirements in the contract are executed on time and with the QA/QC requirements in the system as defined by the contract work plan and in the QAPP. Responsible for verifying that the data produced are of known and acceptable quality.

**SAML** – Texas A&M AgriLife Research – Soil and Aquatic Microbiology Lab, College Station, Texas. Responsible for bacterial source tracking sample analysis, and LU/LC bacteria source assessments.

Terry Gentry, Assoc. Prof. of Soil & Aquatic Microbiology; SAML Director & Project Co-Lead

Responsible for performing bacterial source tracking (BST) analysis and related activities. This includes ensuring that SAML personnel involved in generating analytical data have adequate training and thorough knowledge of the QAPP and all standard operating procedures (SOPs) specific to the analyses or task performed. Responsible for oversight of all SAML operations ensuring that all QA/QC requirements are met, documentation related to the analysis is complete and adequately maintained, and that results are reported accurately. Responsible for ensuring that corrective action is implemented, documented, reported and verified. Monitors implementation of the measures within SAML to ensure complete compliance with project DQOs in the QAPP. Conducts in-house audits to ensure QAPP compliance and identify potential problems.

**IRNR** – Texas A&M Institute of Renewable Natural Resources. Responsible for wildlife population surveys and density estimates conducted in support of LU/LC source assessments, known source sample collection.

Roel Lopez, IRNR Director; IRNR Project Co-Lead

Responsible for overseeing wildlife population surveys and known source fecal sample collection activities. This includes ensuring that field personnel involved in sample and survey data collection have adequate training and thorough knowledge of the QAPP and all SOPs specific to the task performed. Responsible for oversight of fecal sampling tasks ensuring that all QA/QC requirements are met, documentation related to collections is complete and adequately maintained and reported accurately. Responsible for ensuring that corrective action is implemented, documented, reported and verified. Monitors data collection practices to ensure compliance with project DQOs in the QAPP. Conducts field audits to ensure QAPP compliance and identify potential problems.

**USDA-ARS** – United States Department of Agriculture-Agricultural Research Service, Grasslands Soil and Water Research Lab, Temple, Texas. Responsible for site instrumentation, sample collection, and LU/LC source assessment assistance.

Daren Harmel, Agricultural Engineer & Project Co-Lead

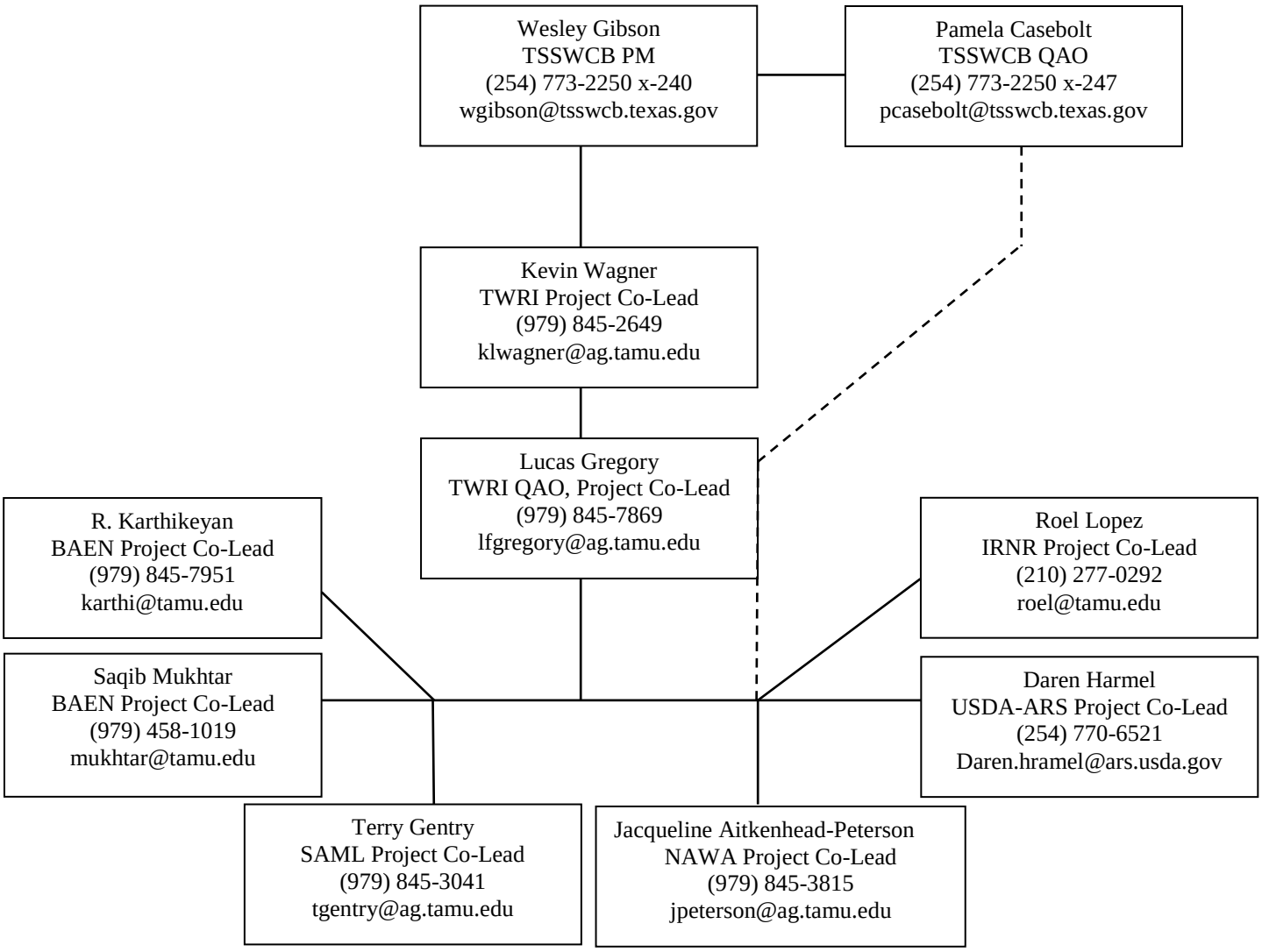
Responsible for overseeing soil, water and known source fecal sample collection activities. This includes ensuring that field personnel involved in sample collection have adequate training and thorough knowledge of the QAPP and all SOPs specific to the task performed. Responsible for oversight of soil, water and known source fecal sampling tasks ensuring that all QA/QC requirements are met, documentation related to collections is complete and adequately maintained and reported accurately. Responsible for ensuring that corrective action is implemented, documented, reported and verified. Monitors implementation of field measures to ensure complete compliance with project DQOs in the QAPP. Conducts field audits to ensure QAPP compliance and identify potential problems.

**NAWA** – Texas A&M AgriLife Research – Nutrient and Water Analysis Lab, College Station, Texas. Responsible for carbon and ortho-phosphorus analysis of water and sediment samples.

Jacqueline Aitkenhead-Peterson, Assistant Professor and Project Co-Lead

Responsible for nutrient analysis on samples collected during simulated instream trials. This includes ensuring that personnel involved in sample analysis have adequate training and thorough knowledge of the QAPP and all SOPs specific to the task performed. Responsible for oversight of nutrient analysis tasks ensuring that all QA/QC requirements are met, documentation related to collections is complete and adequately maintained and reported accurately. Responsible for ensuring that corrective action is implemented, documented, reported and verified. Monitors analysis techniques to ensure complete compliance with project DQOs in the QAPP. Conducts audits to ensure QAPP compliance and identify potential problems.

**Figure A4.1: Project Organization Chart**



# Section A5: Problem Definition/Background

Bacteria impairments have been and continue to constitute the bulk of individual waterbody impairments in the state. As illustrated in the *2010 Texas Water Quality Inventory and 303(d) List*, 621 impairments are documented in Texas and 319 of those are attributed to bacteria. This represents roughly 51% of all impairments in the state. The *2012 Texas Integrated Report* illustrated similar levels of bacteria impairments once approved further emphasizing the need to better understand the sources and fate of bacteria in watersheds so that these impairments can be effectively addressed and managed.

Despite having been studied for several decades, many shortcomings exist in knowledge about fate and transport of *Escherichia coli (E. coli)* in the environment. In freshwater streams, *E. coli* are used as an indicator of fecal material from warm-blooded animals present in the water column. Initial assumptions were that these indicator organisms only existed in the gastrointestinal tracts of warm-blooded animals or their freshly excreted fecal material. This dogma regarding *E. coli's* reliance on the intestinal tract of warm-blooded animals led to its widespread use as an indicator of fecal contamination. Recent work has shown that *E. coli* can persist and grow outside of their host in both soil and water (Bolster et al. 2005; Ishii et al. 2006; Habteselassie et al. 2008; Vital et al. 2008; Garzio-Hadzick et al. 2010; Harmel et al. 2010; Vital et al. 2010) thus jeopardizing their effectiveness as accurate indicators of fecal contamination.

State-wide, watershed managers are currently utilizing tools to predict the sources, population dynamics (i.e. occurrence, growth, persistence), and transport of bacteria and are subsequently planning and implementing management strategies to address bacteria loadings into a waterbody based on these available tools. Given the fact that the sources and fate of *E. coli* in the environment are poorly understood, these tools cannot be expected to accurately illustrate *E. coli* behavior much less be able to accurately illustrate how planned management practices may alter current *E. coli* loadings.

In 2009, the final version of the "Bacterial Total Maximum Daily Load (TMDL) Task Force Report" was published culminating discussions among experts in the field of bacteria related water resources management. This report focused on describing appropriate and cost-effective ways to implement bacteria TMDLs in Texas. Additionally, the report also identified needs for further evaluations to reduce uncertainty about bacteria behavior under varying water conditions in Texas. Largely in response to this report, the "Fate and Transport of *E. coli* in Rural Texas Landscapes and Streams" project (TSSWCB Project 07-06) was developed to begin addressing some of the identified information needs. Results of this work illustrated that the presence, fate and transport of bacteria is highly variable. *E. coli* concentrations varied widely within and between species as did the kinetic growth and decay constants for *E. coli* from each species. Evaluations of *E. coli* from other animal species were identified as a critical need for future watershed bacteria studies. Additionally, environmental factor (temperature and moisture) variations were also found to significantly influence *E. coli* survival and growth. Future work to evaluate fluctuations in nutrient conditions under simulated 'natural' conditions was recommended as a way to evaluate the response of *E. coli* in the water and sediment profiles to changes in stream water quality.

This need for additional investigation is echoed in the "Bacteria TMDL Task Force Report." The report expressly states that "studies to identify dominant environmental factors that affect bacterial transport in streams (e.g., physical and chemical composition of stream waters [pH, total suspended solids, total dissolved solids, nutrients, etc.], temperature, etc.)," are needed to better characterize the kinetic growth and decay rates of bacteria in stream environments. Additionally, the interactions of water and sediment in the stream environment are not clearly defined and need to be better understood. Contributions of wastewater and associated nutrients to stream systems further complicate instream regrowth issues and are pointed to in the "Bacteria TMDL Task Force Report" as a needed area of exploration.

# Section A6: Project/Task Description

Building upon results from TSSWCB Project 07-06 and further focusing on addressing informational needs identified in the "Bacteria TMDL Task Force Report," this project will focus on two primary tasks: 1) evaluating the predominant water quality parameters affecting instream bacterial fate and 2) evaluating and quantifying contributing *E. coli* loading to designated LU/LC types. These specific tasks were selected as those that will provide the most valuable information to watershed managers and practitioners who are faced with accurately predicting and planning to manage *E. coli* loading in Texas Watersheds. This will be supported by education and outreach efforts that deliver project results to personnel at local, regional, state and national levels.

Instream water quality parameters affecting *E. coli* growth and persistence will be quantified utilizing simulated stream environments. Flow chambers will be constructed in a laboratory setting to enable selected environmental parameters to be controlled and manipulated among treatments of simulated instream conditions. Flow chambers will consist of water tight channels fitted with water circulation devices to allow flow to mimic 'natural' low flow conditions. The flow rate can be adjusted to allow for adjustments to the simulated flow conditions. Flow chambers will be filled with stream water collected from Carters Creek near Briarcrest Drive in Bryan, TX under various flow conditions (Figure A6.1). Once in the flow chambers, water will be sampled at prescribed time intervals and will be evaluated to concurrently determine changes in *E. coli*, Dissolved Oxygen (DO), Dissolved Organic Carbon (DOC), pH, specific conductance, nutrient parameters (Ammonium, Nitrate, and Ortho-phosphorus) and turbidity. Individual nutrient parameter spikes will be applied to illustrate the direct impact of each parameter on *E. coli* growth and persistence.

Bacteria source identification will be conducted on multiple LU/LC types monitored at the USDA-ARS Grassland Research Facility in Riesel (Figure A6.2). USDA-ARS will collect 20 surface runoff samples and 25 soil samples from each of 3 established catchments in Riesel. USDA-ARS will remit samples to SAML for *E. coli* enumeration and BST analysis. SAML will utilize the library-dependent ERIC-RP (ERIC-PCR and RiboPrinting combination method) BST technique and compare results to both a local and state-wide BST library to evaluate the sources contributing bacteria to specific LU/LCs. Camera trap arrays will be established on each LU/LC type to monitor and estimate species presence and develop species indices. Known sources of fecal material will be collected, processed and incorporated into the Texas *E. coli* BST Library and utilized in the library-dependent BST analysis to further support bacteria loading identification.

Delivery of project results and findings is a critical last step that will be completed through this project. Information on these topics is in high demand and ample opportunities exist to deliver findings to interested parties through focused workshops, meetings and conferences. One such avenue that results will potentially be presented at is the Texas Watershed Coordinator Roundtable. The July 27, 2011 meeting of this group focused solely on bacteria related content and this was by far the biggest audience at these roundtable events to date. The engagement of the audience clearly illustrated the desired/need for further information on bacteria related topics, especially those that will be addressed through this project. Development of peer-reviewed

publications (or working drafts) is another way that education and outreach will be achieved through this project. Additionally, this provides extra credibility to the work done thus solidifying the significance of the work conducted through this project and enabling it to be more rapidly and widely utilized.

**Table A6.1: Project Plan Milestones**

| Task | Project Milestone | Agency | Start | End |
|---|---|---|---|---|
| 2.1 | Develop QAPP | TWRI | M1 | M4 |
| 2.2 | QAPP Annual Revision and Amendments | TWRI | M4 | M24 |
| 3.1 | Establish lab-scale flow chambers | TWRI & BAEN | M1 | M4 |
| 3.2 | Populate flow chambers with various types of water | TWRI & BAEN | M4 | M20 |
| 3.3 | Conduct replicated monitoring and analysis | TWRI & BAEN | M4 | M20 |
| 3.4 | Evaluate bacteria population dynamics | TWRI & BAEN | M14 | M20 |
| 4.1 | Establish camera arrays to document species presence and indices | IRNR | M4 | M16 |
| 4.2 | Develop fecal loading rates | TWRI & BAEN | M16 | M20 |
| 4.3 | Collect identified sources of fecal material | IRNR & USDA-ARS | M4 | M20 |
| 4.4 | Collect surface water runoff samples | USDA-ARS | M4 | M20 |
| 4.5 | Collect soil samples | USDA-ARS | M4 | M20 |
| 4.6 | Process samples using USEPA Method 1603 | SAML | M4 | M20 |
| 4.7 | Compare *E. coli* isolates from soil and water samples to *E. coli* strains isolated and typed in task 4.3 and others in the Texas *E. coli* BST Library | SAML | M16 | M20 |
| 4.8 | Develop descriptive write up of BST technical approach and its results | SAML | M18 | M21 |
| 5.1 | Deliver project findings | Project personnel | M1 | M24 |
| 5.2 | Maintain project website | TWRI | M1 | M24 |

**Figure A6.1: Carters Creek Watershed**

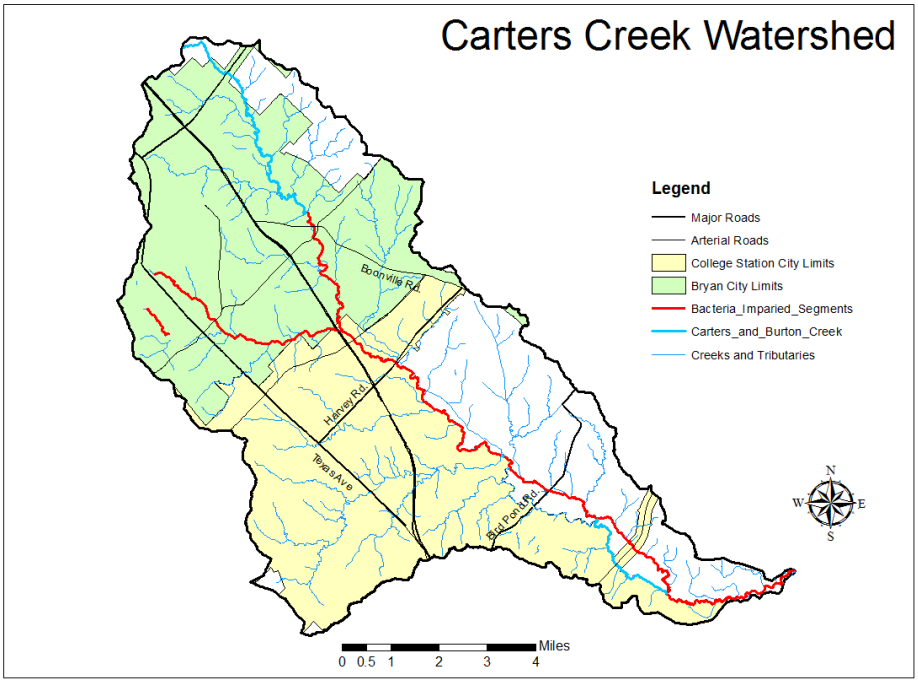
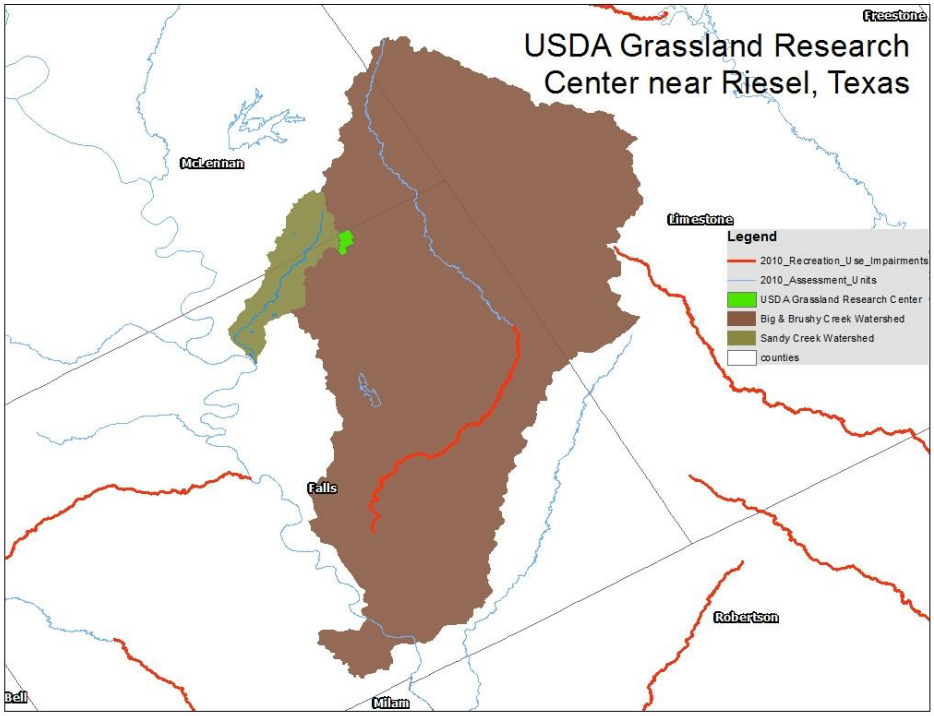



**Figure A6.2: USDA Grassland Research Center near Riesel, TX**



# Section A7: Quality Objectives and Criteria for Data Quality

The objective of this section is to ensure that data collected meets the DQOs of the project. This project has a significant focus on determining background levels of *E. coli* in soil, surface runoff, feces and simulated stream environments. Specific goals of the project that measurement performance specifications are established are:

1. Evaluate the influences of water chemistry and turbidity on *E. coli* growth and persistence in simulated stream environments in the presence of naturally occurring microbial community

2. Evaluate the level and sources of 'background' *E. coli* in soil and surface runoff from three managed LU/LCs that do not have human or livestock derived bacteria contributions using library dependent BST techniques

3. Estimate the presence and local populations of small, meso-, and large mammals by conducting camera and live trapping within each of the three managed LU/LCs

Table A7.1 outlines measurement performance specifications needed to support project goals:

***Ambient Water Reporting Limits (AWRLs)***
The AWRL establishes the reporting specification at or below which data for a parameter must be reported based on given freshwater screening criteria. The AWRLs specified in Table A7.1 are the program-defined reporting specifications for each analyte and yield data of acceptable quality for assessment.

***Precision***
Precision is the degree to which a set of observations or measurements of the same property, obtained under similar conditions, conform to themselves. It is a measure of agreement among replicate measurements of the same property, under prescribed similar conditions, and is an indication of random error.

Laboratory precision is assessed by comparing replicate analyses of laboratory control samples in the sample matrix (e.g. deionized water, sand, commercially available tissue) or sample/duplicate pairs in the case of bacterial analysis. Precision results are compared against measurement performance specifications and used during evaluation of analytical performance. Program-defined measurement performance specifications for precision are defined in Table A7.1.

For quantitative microbiological analyses, the method to be used for calculating precision is the one outlined in *Standard Methods for the Examination of Water and Wastewater*, 21st Edition, section 9020 B.8.b.

$$RPD_{bacteria} = (\log X_1 - \log X_2)$$

Relative percent deviation (RPD) $_{bacteria}$ should be lower than 3.27 ΣRlog/n, where Rlog is the difference in the natural log of duplicates for the first 15 positive samples.

RiboPrinting and enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) BST are qualitative assays, generating two different types of DNA fingerprints. The precision of the ERIC-PCR and RiboPrinting can be measured as the percent of *E. coli* isolates that, when typed multiple times, produce the same ultimate source result in terms of the source identified. Precision for ERIC-PCR and RiboPrinting will be determined using a laboratory control strain of *E. coli* (QC101). For ERIC-PCR and RiboPrinting, the data quality objective is 90% precision. *E. coli* library-dependent PCR based on ERIC-PCR DNA sequences are also currently presence-absence qualitative assays. Precision is determined using fecal DNA or *E. coli* isolate DNA from known human and animal sources with a data quality objective of 100% agreement in marker detection among replicates.

***Accuracy***

Accuracy is a statistical measurement of correctness and includes components of systemic error. A measurement is considered accurate when the result reported does not differ from the true situation. Performance limits are specified in Table A7.1. An additional element of accuracy is the absence of contamination. This is determined through the analysis of blank samples of sterile water processed in a manner identical to the sample. Requirements for blank samples are discussed in Section B5.

Accuracy for BST methods will be assessed using the *E. coli* (QC101) lab control strain for ERIC-RP and fecal DNA or *E. coli* isolate DNA from known human and animal sources for *E. coli* library-dependent PCR. For the ERIC-RP, the data quality objective is 90% accuracy for correct identification to library strain. For *E. coli* library-dependent PCR the data quality objective is 90% accuracy for the presence/absence of the appropriate markers in control DNA samples. An additional element of accuracy is the absence of contamination. This is determined through the analysis of no template controls for the PCR methods.

***Comparability***

The comparability of the data produced is predetermined by the commitment of the staff to use only approved procedures as described in this QAPP. Comparability is also guaranteed by reporting all ambient, library, and QC data for evaluation by others.

***Completeness***

The completeness of the data is a measure of how much of the data is available for use compared to the total potential data. Ideally, 100% of the data should be available. However, the possibility of unavailable data due to accidents, weather, insufficient sample volume, broken or lost samples, etc. is to be expected. Therefore, it will be a general goal of the project(s) that 90 percent data completion is achieved.

## Table A7.1: Data Quality Objectives for Measurement Data

| Parameter | Units | Method Type | Method | Method Description | Parameter Code | AWRL[1] | Precision of Laboratory Duplicates | Accuracy[2] | Percent Complete[3] |
|---|---|---|---|---|---|---|---|---|---|
| *E. coli* RiboPrint fingerprint | NA | DNA/ image matching | SAML SOP | RiboPrinting | NA | NA | 90% identical[4] | 90% correct[4] | 90 |
| *E. coli* ERIC-PCR fingerprint | NA | DNA/ image matching | SAML SOP | ERIC-PCR | NA | NA | 90% identical[4] | 90% correct[4] | 90 |
| *E. coli* in water Enumeration, mTEC | cfu/100mL | Membrane Filter culture on modified mTEC agar | USEPA 1603 (2006) | Membrane Filter | NA | 1 cfu | 3.27*ΣRlog/n | Na | 90 |
| *E. coli* in soil; Enumeration, mTEC | cfu/g dry weight | Membrane Filter culture on modified mTEC agar | USEPA 1603 (2006); Byappanahalli et al. (2003) | Membrane Filter | NA | 1 cfu | 3.27*ΣRlog/n | Na | 90 |
| *E. coli* in feces; Enumeration, mTEC | cfu/g dry weight | Elutriated, Membrane filter culture on modified mTEC agar | USEPA 1603 (2006); Muirhead et al. (2005) | Membrane Filter | NA | 1 cfu | 3.27*ΣRlog/n | Na | 90 |
| Dissolved Oxygen | mg/L | Automated instrument | TCEQ SOP V1 | Rapid pulse sensor | NA | 0 mg/L | NA | 2% or 0.2 mg/L | 90 |
| Dissolved Organic Carbon | mg/L | Shimadzu TOC-V HTCO with chemiluminescent N detection | USEPA 415.1 | Non-purgeable dissolved organic carbone | NA | 0.1 mg/L | <5% CV | ±3% | 90 |
| pH | pH/units | Automated instrument | TCEQ SOP V1 | Guarded glass bulb; standard probe | NA | 0 | NA | 0.2 units | 90 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ammonium | mg/L | Automated colorimetry | EPA 350.1 | Colorimetric | NA | 0.005 mg/L | <5% CV | ±2% | 90 |
| Nitrate | mg/L | Automated colorimetry | EPA 353.2 | Colorimetric | NA | 0.005 mg/L | <5% CV | ±2% | 90 |
| Specific Conductivity | mS/cm | Automated Sensor | SM 4500 O-G | Nikle electrode | NA | 0 ms/cm | NA | 0.5% | 90 |
| Temperature | °C | Automated Sensor | SM 2550 B | high-precision thermistor | NA | -5°C | NA | 0.15°C | 90 |
| Ortho-Phosphorus | mg/L | Colorimetric Automated Ascorbic Acid | USEPA 365.1 | Colorimetric | NA | 0.005 mg/L | <5%CV | ±2% | 90 |
| Turbidity | NTU | Automated Sensor | USEPA 180.1 | Light detector | NA | 0 NTU | NA | ±2% | 90 |
| Soil moisture | % | Gravimetric | Gardner, WH. 1986; Jury et al. 1991 | Direct, oven dried mass balance | NA | NA | NA | NA | 90 |

[1] minimum detection limits for field parameters represent manufacturer specifications and will be used for the AWRL in this instance.
[2] Manufacturer specifications are presented for accuracy limits and method detection limits for field parameters.
[3] The objective is for 90% of the data to be collected.
[4] Accuracy and laboratory method precision for BST will be determined using an *E. coli* QC isolate and DNA from known-source samples

# Section A8: Special Training Requirements/Certifications

Personnel involved in water, soil and feces sampling, sample analyses, and statistical analyses have received the appropriate education and training required to adequately perform their duties and can demonstrate their training as needed. No special certifications are required. Personnel involved in this project have been trained in the appropriate use of field equipment, laboratory equipment, laboratory safety, and all applicable SOPs.

Personnel involved in sample analyses for BST and statistical analyses have received the appropriate education and training required to adequately perform their duties. No special certifications are required. SAML personnel involved in this project have been trained in the appropriate use of laboratory equipment, laboratory safety, cryogenics safety, and all applicable SOPs. Each laboratory analyst must demonstrate their capability to conduct each test that the analyst performs to the Lab Director. This demonstration of capability is performed before analyzing samples and annually thereafter. Finally, SAML is National Environmental Laboratory Accreditation Program (NELAP)-certified for enumerating *E. coli* in both non-potable and drinking water using U.S. Environmental Protection Agency (USEPA) Method 1603.

# Section A9: Documentation and Records

Hard copies of general maintenance records, all field data sheets, chain of custody forms (COCs), laboratory data entry sheets, calibration logs, electronic forms of all project data, and corrective action reports (CARs) will be archived by each laboratory for at least five years. All electronic data will be backed up on an external hard drive monthly and are simultaneously saved in an external network folder and the computer's hard drive. A blank CAR form is presented in Appendix A, a blank COC form is presented in Appendix B, and a blank bacteriological data log sheet is presented in Appendix C.

Quarterly Progress Reports (QPRs) will note activities, items, or areas identified as potential problems and any variations or supplements to the QAPP. CARs will be utilized when necessary. CARs that result in any changes or variations from the QAPP will be made known to pertinent project personnel and documented in an update or amendment to the QAPP. All QPRs and QAPP revisions will be distributed to personnel listed in Section A3.

**Table A9.1 Project Documents and Records**

| Document/Record | Location | Retention | Form |
|---|---|---|---|
| QAPP, amendments, and appendices | TWRI | 5 years | Paper/Electronic |
| Chain of custody records | SAML | 5 years | Paper |
| Sheets of Lading for Fecal Specimens | SAML | 5 years | Paper |
| Corrective action reports | TWRI | 5 years | Paper/Electronic |
| Field notes | IRNR,USDA-ARS | 5 years | Paper |
| Bacteriological data sheet | SAML, BAEN | 5 years | Paper |
| Laboratory QA manuals and/or SOPs | SAML, BAEN | 5 years | Paper/Electronic |
| Lab equipment calibration records & maintenance logs | SAML, BAEN | 5 years | Paper |
| Lab data reports/results | SAML, BAEN | 5 years | Paper/Electronic |
| Quarterly progress reports/final report/data | TWRI | 5 years | Paper/Electronic |

The TSSWCB may elect to take possession of records at the conclusion of the specified retention period.

***QAPP Revision and Amendments***

Until the work described is completed, this QAPP shall be revised as necessary and reissued annually on the anniversary date, or revised and reissued within 120 days of significant changes, whichever is sooner. The last approved version of QAPPs shall remain in effect until a revised version has been fully approved; the revision must be submitted to the TSSWCB for approval before the last approved version has expired. If the entire QAPP is current, valid, and accurately reflects the project goals and the organization's policy, the annual re-issuance may be done by a certification that the plan is current. This will be accomplished by submitting a cover letter station the status of the QAPP and a copy of new, signed approval pages for the QAPP.

QAPP amendments may be necessary to reflect changes in project organization, tasks, schedules, objectives and methods; address deficiencies and non-conformances; improve operational efficiency; and/or accommodate unique or unanticipated circumstances; Written requests for amendments are directed from the TWRI Project Leader or designee to the TSSWCB PM and are effective immediately upon approval by the TSSWCB PM and QAO. Amendments to the QAPP and the reasons for the changes will be documented and distributed to all individuals on the QAPP distribution list by the TWRI Project Leader or designee. Amendments shall be reviewed, approved, and incorporated into a revised QAPP during the annual revision process.

# Section B1: Sampling Process Design (Experimental Design)

***E. coli* Source Assessment**

A source assessment will be conducted on three designated catchments (SW12: un-grazed rangeland, Y6: cropland, SW17: managed hay pasture) at the USDA-ARS Grassland Research Center in Riesel to attempt to determine what the sources of *E. coli* are in these land use types unimpacted by anthropogenic sources. A combined approach that documents wildlife present, collects fecal material from these species and compares *E. coli* isolated from these species to those found in soil and surface runoff samples.

The presence of wildlife species in the study area will be evaluated using motion triggered, infrared game camera trap arrays and Tomahawk traps to identify small and meso-mammals present. Camera trapping will be utilized to document the relative abundance of each identified meso-mammal species within the surveyed landuses. Camera trap arrays and Tomahawk traps will be deployed twice during the study; once in the winter and once in the summer, to illustrate potential differences landuse utilization by species between seasons. Fecal material will also be collected from animals that are physically trapped and will be sent to SAML in College Station for inclusion in the Texas *E. coli* BST Library. Once cultured, 2 *E. coli* isolates from each sample will be processed using the ERIC-RP methodology. Additionally, an *E. coli* count per gram of dry feces will be calculated as well.

To attempt to identify the sources of *E. coli* present in the soils and surface runoff leaving the surveyed sites, soil and surface runoff samples will also be collected from each site. Each sample will be sent to SAML in College Station where *E. coli* will be enumerated (cfu/100mL for water; cfu/g dry wt. for soil) using the USEPA 1603 method. Isolates from each sample (5 per water sample; 4 per soil sample) will be analyzed using ERIC-RP and compared to the Texas *E. coli* BST Library. Once analysis is complete, BST results from soil and surface runoff samples will be compared to results from known source samples collected on site and those included in the Texas *E. coli* BST Library.

**Table B1.1: *E. coli* Source Assessment Monitoring Sites and Frequencies**

| Monitoring Type | Sampling Location | Frequency | Planned # of Samples |
|---|---|---|---|
| Camera Trapping | SW12, SW17, Y6 | 1 summer and 1 winter campaign | NA |
| Physical Trapping | SW12, SW17, Y6 | 1 summer and 1 winter campaign | 50 |
| Fecal Sampling | SW12, SW17, Y6 | continuous | 50 |
| Surface Runoff | SW12, SW17, Y6 | continuous | 60; 20 from each LU/LC |
| Soil | SW12, SW17, Y6 | continuous | 75; 25 from each LU/LC |

**Instream *E. coli* Growth and Persistence Assessment**

The impacts of water quality on instream *E. coli* growth, persistence, die-off, and decay will be evaluated in a simulated stream environment. Five water tight raceways will be used to establish laboratory based flow chambers. A series of four trials will be conducted in which the flow chambers will be filled with unaltered water collected from Carters Creek near Briarcrest Dr. in Bryan, TX, Brazos County. Water chemistry parameters including dissolved oxygen (DO), dissolved organic carbon (DOC), pH, specific conductance, nutrient parameters (ammonium, nitrate, and ortho-phosphorus) and turbidity will be monitored during trials. Baseline water quality will be established at the beginning of each trial. Nutrient treatments that represent a spike in nutrients available in the water column will be developed based on nutrient levels in the baseline assessment. These treatments will be applied to four of the five flow chambers. Flow chambers will be monitored over time using automated instrumentation and grab sampling techniques to evaluate the response of *E. coli* in a natural environment (competition and predation from the microbial community present) to additional nutrients.

An initial screening test will be utilized to evaluate *E. coli* response time to nutrient spikes and will be used to establish planned sampling dates. For example, the screening test may illustrate that *E. coli* growth, persistence and die-off occur within 10 days of the nutrient spike being applied. This would suggest that extending sampling out for 30 days is excessive and we would therefore establish a sampling regime that would collect 10 samples of 14 days rather than 30 days as a result.

Table B1.2 illustrate the sampling frequency, water type to be analyzed, flow rate employed and the season that creek water was collected in.

**Table B1.2: Instream *E. coli* Growth and Persistence Sampling Schedule (all 4 trials)**

| Trial | Sample Dates (days since trial start) | Water Type | Flow Rate | Season |
|---|---|---|---|---|
| 1 | TBD pending screening test | Base | Normal | Summer |
| 2 | TBD pending screening test | Storm | High | Summer |
| 3 | TBD pending screening test | Base | Normal | Winter |
| 4 | TBD pending screening test | Storm | High | Winter |

# Section B2: Sampling Method Requirements

### *E. coli* Source Assessment

This portion of the project focuses on identifying the sources of *E. coli* present in three designated catchment areas (SW12: ungrazed native rangeland; Y6: cropland, and SW17: managed hay pasture) and will involve conducting wildlife surveys on each landuse type in one winter season and one summer season. Additionally, surface runoff and soil samples will be collected and subjected to BST analysis. Results will be compared to those of known source samples collected from these three sites. The goal of this assessment is to identify the sources contributing *E. coli* to these land uses which are free of anthropogenic sources of *E. coli* contribution.

### *Wildlife Survey: Cameras*

IRNR set up arrays of remote digital cameras in the study area on each evaluated land use for 14-21 consecutive days during study seasons. Random locations for cameras will be decided by overlaid grid. Previous research suggests that certain species of meso-mammals (i.e., raccoons [*Procyon lotor*], opossums [*Didelphys virginiana*]]) generally have high population densities and are carriers. Research has been conducted to ascertain the fecal deposition rates of these species (Rowland et al. 1984, Acevedo et al. 2006). Based on an extensive literature review, infrared triggered cameras will be used to aid in determining relative abundances of meso-mammals present (Trolle 2003). Random sample points will be selected and remotely-operated infrared digital cameras will be situated in these locations for at least 14 consecutive days during study seasons. Cameras will be placed at observed wildlife trails or openings suitable for camera placement (Claridge et al. 2004, Trolle and Kéry 2005).

### *Wildlife Survey: Trapping*

IRNR will trap small mammals using randomly located Tomahawk type trap arrays to capture mice, rats and other small creatures (e.g., species less attracted to baits). Relative abundances of trapped species will be calculated based on trap success (Geier and Best 1980, Fryxell et al. 1998). The goal of this trapping is to collect adequate fecal material from 50 animals to process using BST techniques and to incorporate these species into the Texas *E. coli* BST Library.

Animals will be released safely and once clear of the area, technicians will collect approximately 30 grams of feces (Table B2.1). After releasing animals from the trap and collecting a fecal sample, the cage will be cleaned and moved to prevent possible cross contamination of subsequent fecal samples. Traps will be closed every morning and reopened every evening during each trap session to prevent animals from being confined in cages in daylight hours. Traps will be set in shaded areas to reduce heat stress on the animals and for their safety. During periods of high temperature, trapping may be rescheduled.

### *Wildlife Survey: Fecal Collection and Handling*

All collection and handling of fecal specimens conducted by IRNR and USDA-ARS will be performed using all safety precautions (wearing protective gear such as nitrile gloves will be strictly enforced). Specimens will be handled aseptically to ensure sample quality and minimize

exposure of personnel to pathogens. All fecal material and waste collected will be placed in screw capped sterile containers (Table B2.1). Containers will be labeled with: Name of collector, date, and species before collection. Fecal specimens will be placed in an insulated cooler and transported to the SAML Lab in College Station. A goal of 50 known source fecal samples has been set for the project. Additionally, 30 grams of fecal material is the goal weight of feces to be collected from each animal. Should 30 grams not be achievable due to animal size or other factors, as much fecal matter as possible will be collected. At least 0.5 grams of feces must be collected to be useful for *E. coli* enumeration and isolation.

Fecal sample collections are described in Appendix C-1. To ensure fresh samples of known origin, fecal samples will be obtained using one of three methods: a) collected from animals visually observed defecating by technician; b) collected from cages of trapped animals; c) collected from intestines of animals. All fecal samples will be shipped to SAML for BST analysis within 3 days of collection. All fecal material will be processed for bacterial isolation within 24 hours of receipt in the lab.

***Surface Water Runoff Sampling Requirements***

Storm event runoff water samples will be collected using refrigerated ISCO® Avalanche samplers collecting from H-flumes situated at the outlet of each monitored LU/LC type. Flow-weighted composite samples will be collected into clean polyethylene 5-gallon square bottles for runoff events with more than 1.32 mm of runoff volume. Water level in the flume, date, time, and collector's name will be recorded at the time the sample is retrieved from the sampler. Samples will continue to be collected based on a flow-weighted average until samples are collected by field personnel, the single sterile sample bottle is full, or stream depth returns to pre-event levels. After the first sample is collected until the completion of the running program, the Avalanche cools the refrigerated compartment to 1°C ± 1. One hour after the last sample of the program is taken, the Avalanche adjusts its control to maintain the samples at 3°C ± 1. These temperatures are both within the <10°C temperature requirement for non-regulatory storm samples. The 5 gal collection bottle will be removed from the sampler and transported to the laboratory on ice. A new (clean, sterilized sampling bottle) will be placed in the sampler in preparations for the next sampling event. Flow from each watershed site will be measured with a bubble flow meter and established stage-discharge relationship. This, in combination with the EMCs, will allow calculation of bacteria loading for each runoff event. Flow and precipitation data are downloaded at least monthly using an ISCO® 581 Rapid Transfer Device.

Adherence to prescribed holding times as listed in Table B2.1 is achieved through automated notifications. When sampling is initiated, field staff is notified by phone that the sampling unit(s) has been activated. At that point, field staff begins planning to retrieve samples and deliver them to the laboratory within the prescribed holding time.

***Runoff Event Holding Time***

The runoff samples in the 5-gallon bottles will be retrieved from the refrigerated ISCOs, thoroughly mixed, and sub-samples transferred to appropriate containers as outlined in Table B2.1, and transported on ice to the SAML lab for analysis of *E. coli*. The beginning of a storm event is defined as the point in time that flow exceeds the enable levels and the end of the storm

is when flow is below the enable level and when more rain (flow increases) is not expected within 2 hours. At the end of the storm the storm sample should be collected, data downloaded, and ISCO® reset for the next event.

For *E. coli* sub-samples, a minimum volume of 100 ml collected by automatic samplers will be poured into sterile plastic bottles and stored in refrigeration at 4°C. Edge-of-field *E. coli* samples must be removed from automatic samplers, transported to the SAML laboratory, filtered, and placed in the incubator within 24 hours of the start of the stormwater runoff event, that is, from the first automatically collected stormwater sample. This applies even when storm events exceed 24 hours (although not expected due to the small size of the drainage areas involved).

All samples will be transported at 4°C to the lab(s) for analysis. All filtration and incubation will be performed in the laboratory. Samples must be stored at 4°C until processed in each lab. In the event that *E. coli* samples cannot be collected, transported, processed and incubated within 24 hours, samples will still be analyzed but it will be noted that the target holding time was not met.

***Soil Sample Collection***

Soil samples will be collected seasonally within each LU/LC plot to evaluate the sources of *E. coli* present in the soil and the relative abundance of *E. coli* in the soil a per dry gram basis. Sampling will be conducted along a grid. At least 30 g of soil sample is required for routine analyses. Sampling of areas such as small gullies, slight field depressions, terrace waterways, or unusual areas will be avoided.

A total of 25 soil samples will be collected from each of the three LU/LCs monitored at the USDA-ARS Grassland Research Facility near Riesel. Samples will be collected to a depth of 6 cm using a standard soil auger following the removal of surficial organic matter or debris (leaves, grass blades, twigs, etc.) from the soil surface. Care will be taken to not remove the O horizon of the soil column.

**Instream *E. coli* Growth and Persistence Assessment**

This part of the project will evaluate the growth, persistence, die-off and decay of *E. coli* in simulated stream environments in response to changes in water quality over time. Three flow chambers will be established in the BAEN laboratory and will be filled with unaltered stream water collected from Carters Creek. One flow chamber will be used as a control while the other two will be used as treatments during each trial.

***Water Collection and Flow Chamber Filling***

Water from Carters Creek, upstream of wastewater treatment plant influences, will be collected in clean, 5 gallon sample containers. Once collected, water will be immediately returned at its ambient temperature to the BAEN lab and placed into flow chambers. Water will not be chilled as the travel time from the collection location to the lab is less than 30 minutes and the water will be allowed to remain at ambient temperatures once in the flow chambers. Prior to filling, flow chambers will be washed with bleach water, rinsed with tap water and double rinsed with distilled water. An equal volume of creek water will be placed into each of the flow chambers.

***Water Sample Collection***

Water samples collected from the flow chambers that will be analyzed for *E. coli,* DOC and ortho-phosphorus will be collected directly into clean high-density polyethylene (HDPE) containers. Circulation pumps within the flow chambers will be equipped with a sampling tube that allows the flow of the system to be directed to the sample container. This will reduce the potential for contaminants to be introduced to the flow chamber during sampling.

Once collected, samples will be immediately placed into a refrigerator to begin cooling down to 4°C. Table B2.1 illustrates required container types, preservation needs, temperature thresholds, sample sizes and holding time requirements for samples collected from water.

**Table B2.1: Container Types, Preservation Requirements, Temperature, Sample Size, and Holding Time Requirements.**

| Parameter | Matrix | Container | Preservation | Temperature | Sample Size | Holding Time |
|---|---|---|---|---|---|---|
| ***E. coli* Source Assessment Samples** | | | | | | |
| *E. coli* | water | sterile plastic bag | none | 4°C | 125 mL | 24 hours[1] |
| *E. coli* | soil | sterile plastic bottle | none | 4°C | 30 g | 24 hours |
| Fecal specimen | feces | sterile plastic bottle | none | 4°C | 30 g[2] | 48 hours |
| **Instream *E. coli* Response Samples** | | | | | | |
| Dissolved Organic Carbon | water | HDPE | N/A | 4°C | 100 mL | 24 hours |
| Ortho-Phosphorus | water | HDPE | N/A | 4°C | 100 mL | 24 hours |
| Ammonium | water | HDPE | N/A | 4°C | 100 mL | 24 hours |
| Nitrate | water | HDPE | N/A | 4°C | 100 mL | 20 hours |
| *E. coli* | water | Sterilized HDPE | N/A | 4°C | 125 mL | 8 hours |

[1] 24 hours to processing in laboratory for non-regulatory samples. In the case that the 24-hour holding time is not met, the *E. coli* quantitative count will be flagged

[2] 30 grams is the goal weight for fecal matter collection; however, should it not be possible to collect 30 grams of feces, as much material as possible will be collected. 0.5 grams is the minimum allowable weight of fecal material to be collected

***Processes to Prevent Cross Contamination***

To prevent cross-contamination, stormwater subsamples will be transferred directly from the 5-gallon sampler bottle into the containers they will be transported to the appropriate lab in while

grab samples will be collected directly into containers they will be transported to the lab in. Soil samples will be collected with cleaned probes/shovels into clean 5 gallon buckets for mixing. Probes and buckets are wiped with a cloth then "washed" with ambient soil from the next site to ensure that all soil residue from the previous site has been removed. Soil subsamples will be placed into new plastic bags for transport to labs. Field QC samples as discussed in Section B5 are collected to verify that cross-contamination has not occurred.

***Documentation of Field Sampling Activities***

***Recording Data***

For the purposes of this section and subsequent sections, all field and laboratory personnel follow the basic rules for recording information as documented below:

- Legible writing with no modifications, write-overs or cross-outs;
- Correction of errors with a single line followed by an initial and date;
- Close-outs on incomplete pages with an initialed and dated diagonal line.

Each fecal sample will be collected aseptically in a new, sterile fecal tube (Sarstedt, cat# 80.734.311). Specimen tubes will be labeled with:

a. Sampling date
b. Sampling time
c. Animal species
d. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
e. Sample collector's name, initials
f. Any other pertinent information, e.g., sex of animal; juvenile or adult

All the sample information will be logged into a field log. Samples should be refrigerated (~4°C) or kept on ice following collection and shipped to the SAML laboratory on ice within 3 days of collection. See SOP in Appendix C for complete protocol.

Safety is an issue when working with fecal samples due to the bacterial concentration. Hazardous material safety handling instructions will be included in a file for driver to carry that will be visible on seat or dash of vehicle in case of accident or being stopped by law enforcement officers. Biohazard signs will be placed on the cooler containing samples collected for transport. Sheets of Lading (Appendix B) will be on hand with the field technician and completed for each fecal sample collected along with a COC form.

# Section B3: Sample Handling and Custody Requirements

***Chain-of-Custody***

Proper sample handling and custody procedures ensure the custody and integrity of samples beginning at the time of sampling and continuing through transport, sample receipt, preparation, and analysis. The COC form is used to document sample handling during transfer from the field to the laboratory and inter-laboratory. The sample number, location, date, changes in possession and other pertinent data will be recorded in indelible ink on the COC. The sample collector will sign the COC and transport it with the sample to the laboratory. At the laboratory, samples are inventoried against the accompanying COC. Any discrepancies will be noted at that time and the COC will be signed for acceptance of custody. Sample numbers will then be recorded into a laboratory sample log, where the laboratory staff member who receives the sample will sign it. A copy of a blank COC form used on this project is included as Appendix B.

***Sample Labeling***

Samples will be labeled on the container with an indelible, waterproof marker. Label information will include site identification, date, sampler's initials, and time of sampling. The COC form will accompany all sets of sample containers.

***Sample Handling***

Following collection, samples will be placed on ice in an insulated cooler for transport to the laboratory. At the laboratory, samples will be placed in a refrigerated cooler dedicated to sample storage. The Laboratory Supervisor has the responsibility to ensure that holding times are met with fecal samples. The holding time is documented on the COC. Any problem will be documented with a CAR.

***Failures in Chain-of-Custody and Corrective Action***

All failures associated with COC procedures are to be immediately reported to the TSSWCB PM. Failures include such items as delays in transfer, resulting in holding time violations; violations of sample preservation requirements; incomplete documentation, including signatures; possible tampering of samples; broken or spilled samples, etc. The Project Leader and the TSSWCB PM/QAO will determine if the procedural violation may have compromised the validity of the resulting data. Any failure that potentially compromises data validity will invalidate data, and the sampling event should be repeated. CARs will be reported to the TSSWCB in the QPR. The CARs will be maintained by the TWRI Project Lead.

# Section B4: Analytical Method Requirements

## *E. coli* Source Assessment

### *Fecal Sample Preparation and Processing*

*E. coli* will be extracted from fecal samples upon arrival at the lab to assess initial *E. coli* levels. *E. coli* will be extracted from the fecal samples by placing 1 g of sample in 99 mL of deionized (DI) water. Serial dilutions of the fecal sample and water solution will then be prepared (as needed) and analyzed by laboratory personnel using modified mTEC agar, USEPA Method 1603 [EPA-821-R-06-011. July 2006. *Escherichia coli* in Water by Membrane Filtration Using Modified Membrane-Thermotolerant *Escherichia coli* (modified m-TEC) Agar].

Samples collected by IRNR, USDA-ARS and project partners and sent to SAML will be streaked (resuspended in buffer if necessary) onto modified mTEC medium. Modified mTEC medium is used in USEPA Method 1603 for water samples. Its use for source samples helps avoid selection of different types of *E. coli* due to different media. The modified medium contains the chromogen 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (Magenta Gluc), which is catabolized to glucuronic acid (a red/magenta-colored compound) by *E. coli* that produces the enzyme β-D-glucuronidase. This enzyme is the same enzyme tested for using other substrates such as the fluorogenic reaction with 4-methylumbelliferyl-β-D-glucuronide (MUG) observed by ultraviolet light (UV) fluorescence. Inoculated plates will be incubated at 35±0.5$^{o}$C for 2 hours to resuscitate stressed bacteria and then incubated at 44.5±0.2$^{o}$C for approximately 20 to 24 hours.

### *Soil Sample Preparation and Processing*

*E. coli* will be extracted from soil samples upon arrival at the lab by placing 1 g of sample in 99 mL of DI water. Serial dilutions of the sediment and water solution will then be prepared and analyzed using USEPA Method 1603 as described above.

### *Surface Runoff Sample Preparation and Processing*

*E. coli* in water samples will be isolated and enumerated by laboratory personnel using modified mTEC agar, USEPA Method 1603 as described above. All laboratory sampling areas and equipment (incubator and filtering apparatus) will be sterilized with at least one or in any combination of the following methods--ethyl alcohol, bleach, ultra-violet (UV) light, or autoclave. All disposables will be placed in a heat-resistant biohazard bag and autoclaved prior to disposal.

### *BST Analysis*

The analytical methods utilized in BST analysis and sample preparation are listed in Table B4.1 and Table A7.1 and described in detail in Appendix C. All laboratory sampling areas and equipment will be sterilized with at least one or in any combination of the following methods: ethyl alcohol, bleach, UV light, or autoclave. All disposables will be placed in a heat-resistant biohazard bag and autoclaved prior to disposal.

Fecal specimens, surface water runoff, and soil samples collected by IRNR, USDA-ARS and project partners and sent to SAML for BST analysis will all be processed using USEPA Method 1603 as described above.

*E. coli* colonies from the modified mTEC medium will be picked and streaked for purity on nutrient agar with MUG (NA-MUG) to confirm glucuronidase activity and culture purity for the source sample isolates. Cultures of selected isolates will be archived using glycerol freezing medium (-80$^{o}$C). Two isolates per sample will be analyzed by ERIC-RP per known source fecal samples, five isolates will be analyzed per surface water runoff sample, and four isolates will be analyzed per soil sample.

Three confirmed *E. coli* bacterial colonies from each source sample will be screened for clones (identical strains from the same sample) using a repetitive sequence polymerase chain reaction (rep-PCR) method. ERIC-PCR, a type of rep-PCR, has moderately high ability to resolve different closely related bacterial strains. Consumable costs for ERIC-PCR are inexpensive and labor costs for sample processing and data analyses are moderate. ERIC-PCR is a genetic fingerprinting method used in previous BST studies as well as many microbial ecology and epidemiological studies. ERIC elements are repeat DNA sequences found in varying numbers and locations in the genomes of different bacteria such as *E. coli*. The PCR is used to amplify the DNA regions between adjacent ERIC elements. This generates a DNA banding pattern or fingerprint which looks similar to a barcode pattern. Different strains of *E. coli* bacteria have different numbers and locations of ERIC elements in their bacterial genomes, and therefore, have different ERIC-PCR fingerprints. ERIC-PCR is useful as a screening technique for library development because of its moderate cost and moderately high ability to resolve different strains of the same species of bacteria. Though rep-PCR banding patterns for isolates tend to be generally stable, differences in fingerprint image processing and PCR protocols between laboratories may result in reduced between-laboratory reproducibility and pose a challenge to generating a composite library in multiple laboratories. Rigorous QA/QC, standardized protocols for PCR and image processing, and adequate training of personnel is crucial for generation of comparable data.

Non-clonal isolates will then be RiboPrinted. Ribotyping is a genetic fingerprinting method used in previous BST studies and many microbial ecology and epidemiological studies. In general, an endonuclease enzyme (*Hind* III) selectively cuts *E. coli* DNA wherever it recognizes a specific DNA sequence. The resulting DNA fragments are separated by size and probed for fragments containing particular conserved ribosomal RNA gene sequences, which results in DNA banding patterns or fingerprints that look similar to barcodes. Different strains of *E. coli* bacteria have differences in their DNA sequences and different numbers and locations of enzyme cutting sites, and therefore have different ribotyping fingerprints. The DuPont Qualicon RiboPrinter Microbial Characterization System allows automation of the ribotyping ('RiboPrinting').

After screening the ERIC-RP fingerprints of these known source samples for host specificity, they will be included in the Texas *E. coli* BST Library of *E. coli* isolates from known animal and human sources collected throughout Texas.

**Instream *E. coli* Growth and Persistence Assessment**

*E. coli* in water samples will be isolated and enumerated by laboratory personnel using modified mTEC agar, USEPA Method 1603 as described previously. All laboratory sampling areas and equipment (incubator, filtering apparatus, laminar flow hood) will be sterilized with at least one or in any combination of the following methods--ethyl alcohol, bleach, UV light, or autoclave. All disposables will be placed in a heat-resistant biohazard bag and autoclaved prior to disposal. Aseptic techniques will be followed throughout all analyses to ensure that sample cross contamination does not occur.

Water samples will also be analyzed for DO, DOC, pH, ammonium, nitrate, ortho-phosphorus, and turbidity. With the exception of nitrate, ammonium, DOC, ortho-phosphorus, and turbidity, these parameters will be measured with automated instrumentation listed in Table B4.1.

Nitrate-N in water samples will be analyzed in the NAWA lab using USEPA Method 353.2 [Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry, Method 353.2; USEPA 353.2-1. 1993]. This method determines nitrate-N singly or nitrate-N and nitrite-N combined. Nitrate-N is determined colorimetrically by subtracting the nitrate reading from the nitrite-N reading which is produced from nitrate reduced to nitrite by passing through a copper-cadmium granule column.

Ammonium-N in water samples will be analyzed in the NAWA lab using USEPA Method 350.1 [Determination of Ammonium Nitrogen by Semi-Automated Colorimetry, Method 350.1; USEPA 350.1-1. 1993]. This method determines ammonium-N by buffering and distilling the sample into boric acid which forms a blue color that is measured colorimetrically.

Ortho-phosphorous-P in water samples will be analyzed in the NAWA lab using USEPA Method 365.1 [Phosphorous, All Forms. Method 365.1 (Colorimetric, Automated, Ascorbic Acid). Pp.365-1.1 – 365-1.7. In *Methods for Chemical Analysis of Water and Wastes.* EPA-600/ 4-79-020. 1983.]. Ortho-phosphorous-P concentrations are determined through the ascorbic acid, molybdate blue method.

Dissolved organic carbon in water samples will be analyzed in the NAWA lab using USEPA Method 415.1 [Total Organic Carbon in Water. USEPA Method 415.1 (Combustion or Oxidation)]. This method measures organic carbon concentrations in drinking water, surface and saline waters, domestic and industrial wastes and is most applicable to organic carbon levels above 1 mg/L. Organic carbon in the sample is converted to carbon dioxide ($CO_2$) by catalytic combustion or wet chemical oxidation. $CO_2$ formed can be measured directly by and infrared detector or converted to methane ($CH_4$) and measured by flame detector. The amount of $CO_2$ or $CH_4$ is directly proportional to the concentration of carbonaceous material in the sample.

**Table B4.1: Laboratory Analytical Methods**

| Laboratory Parameter | Method | Equipment Used |
|---|---|---|
| *E. coli* ERIC-PCR fingerprint | SAML SOP | PCR thermal cycler, gel electrophoresis app |
| *E. coli* RiboPrint fingerprint | SAML SOP | RiboPrinter |
| *E. coli* in feces, soil, water | USEPA 1603 | Filtration apparatus, incubator |
| Ortho-phosphorous | USEPA 365.1 | Unity Scientific, SmartChem 200 |
| Dissolved Organic Carbon | USEPA 415.1 | Shimadzu TOC-$V_{CSH}$ & TNM-11 |
| pH | USEPA 150.2 | YSI 6561 |
| Dissolved Oxygen | SM 4500-O G | YSI 6562 |
| Specific Conductance | USEPA 120.1 | YSI 6560 |
| Temperature | SM 2550-B | YSI 6560 |
| Ammonium | USEPA 350.1 | Unity Scientific, SmartChem 200 |
| Nitrate | USEPA 353.2 | Unity Scientific, SmartChem 200 |
| Turbidity | USEPA 180.1 | Hach HI 83414 |

SOP = Standard Operating Procedure

**Failures in Measurement Systems and Corrective Actions**

Failures in measurement systems involve, but are not limited to such things as instrument malfunctions, failures in calibration, blank contamination, QC samples outside QAPP defined limits, etc. In many cases, the field technician or lab analyst will be able to correct the problem. If the problem is resolvable by the field technician or lab analyst, then they will document the problem on the field data sheet or laboratory record and complete the analysis. If the problem is not resolvable, then it is conveyed to the SAML Director, who will make the determination in coordination with the TWRI QAO. If the analytical system failure may compromise the sample results, the resulting data will not be reported to the TSSWCB as part of this project. The nature and disposition of the problem is reported on the data report. The TWRI QAO will include this information in the CAR and submit with the QPR which is sent to the TSSWCB PM.

**Standards Traceability**

All standards used in the field and laboratory are traceable to certified reference materials. Standards preparation is fully documented and maintained in a standards log book. Each documentation includes information concerning the standard identification, starting materials, including concentration, amount used and lot number; date prepared, expiration date and preparer's initials/signature. The reagent bottle is labeled in a way that will trace the reagent back to preparation.

# Section B5: Quality Control Requirements

Table A7.1 in Section A7 lists the required accuracy, precision, and completeness limits for the parameters of interest. Specific requirements are summarized in Table B5.1 and described below. It is the responsibility of the Project Leader to verify that the data are representative. The Project Leader also has the responsibility of determining that the 90% completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents requiring corrective action will be documented through use of CARs (Appendix A). Laboratory audits, sampling site audits, and quality assurance of field sampling methods will be conducted by the TSSWCB QAO or their designee.

### Table B5.1. Required Quality Control Analyses

| Parameter | Matrix | LOQ | LOQ Check Std | LCS | Lab Dup | Field Blank | Method Blank |
|---|---|---|---|---|---|---|---|
| ***E. coli* Source Assessment Samples** | | | | | | | |
| *E. coli* ERIC-PCR | *Feces, Soil, Water* | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* |
| *E. coli* RiboPrint | *Feces, Soil, Water* | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* |
| *E. coli* | *Feces, Soil, Water* | *NA* | *NA* | *NA* | √ | *NA* | √ |
| **Instream *E. coli* Growth and Persistence Assessment Samples** | | | | | | | |
| *Total Inorganic N* | *Soil* | *NA* | *NA* | *NA* | *NA* | *NA* | *NA* |
| *Ortho- Phosphorus* | *Water* | √ | √ | √ | √ | √ | √ |
| *Dissolved Organic Carbon* | *Water* | √ | √ | √ | √ | √ | √ |
| *Ammonium-Nitrogen* | *Water* | √ | √ | √ | √ | √ | √ |
| *Nitrate-Nitrogen* | *Water* | √ | √ | √ | √ | √ | √ |
| *E. coli* | *Feces, Soil, Water* | *NA* | *NA* | *NA* | √ | *NA* | √ |

## Instream *E. coli* Growth and Persistence Assessment

### Method Specific QC requirements

QC samples, other than those specified later this section, are run (e.g., sample duplicates, surrogates, internal standards, continuing calibration samples, interference check samples, positive control, negative control, and media blank) as specified in the methods. The requirements for these samples, their acceptance criteria or instructions for establishing criteria, and corrective actions are method-specific.

**Limit of Quantitation (LOQ)**
The laboratories will analyze a calibration standard (if applicable) at the LOQ on each day samples are analyzed. Calibrations including the standard at the LOQ will meet the calibration requirements of the analytical method or corrective action will be implemented.

**LOQ Check Standard**
An LOQ check standard consists of a sample matrix (e.g., deionized water, sand, commercially available tissue) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system at the lower limits of analysis. The LOQ check standard is spiked into the sample matrix at a level less than or near the LOQ for each analyte for each batch of samples are run.

LOQ check standards are carried throughout the preparation and analytical process and are run at a rate of one per analytical batch. A batch is defined as samples that are analyzed together with the same method and personnel, using the same lots of reagents. The percent recovery of the LOQ check standard is calculated using the following equation in which %R is percent recovery, SR is the sample result, and SA is the reference concentration for the check standard:

$$\%R = SR/SA * 100$$

Measurement performance specifications are used to determine the acceptability of LOQ Check Standard analyses as specified in Table A7.1.

**Laboratory Control Sample (LCS)**
An LCS consists of a sample matrix (e.g., deionized water, sand) free from the analytes of interest spiked with verified known amounts of analytes or a material containing known and verified amounts of analytes. It is used to establish intra-laboratory bias to assess the performance of the measurement system. The LCS is spiked into the sample matrix at a level less than or near the midpoint of the calibration for each analyte. The LCS is carried through the complete preparation and analytical process. LCSs are run at a rate of one per analytical batch. Results of LCSs are calculated by percent recovery, which is defined as 100 times the measured concentration, divided by the true concentration of the spiked sample. The following formula is used to calculate percent recovery, where %R is percent recovery; SR is the measured result; and SA is the true result:

$$\%R = SR/SA * 100$$

Measurement performance specifications are used to determine the acceptability of LCS analyses as specified in Table A7.1.

**Laboratory Duplicates**
A laboratory duplicate is prepared by taking aliquots of a sample from the same container under laboratory conditions and processed and analyzed independently. A laboratory control sample duplicate (LCSD) is prepared in the laboratory by splitting aliquots of an LCS. Both samples are carried through the entire preparation and analytical process. LCSDs are used to assess precision

and are performed at a rate of one per batch. A batch is defined as samples that are analyzed together with the same method and personnel, using the same lots of reagents. For most parameters, precision is calculated by the relative percent difference (RPD) of LCS duplicate results as defined by 100 times the difference (range) of each duplicate set, divided by the average value (mean) of the set. For duplicate results, $X_1$ and $X_2$, the RPD is calculated from the following equation:

$$RPD = (X_1 - X_2)/\{(X_1+X_2)/2\} * 100$$

Bacteriological duplicates are a special type of laboratory duplicate. Bacteriological duplicate analyses are performed on samples from the sample bottle on a 10% basis. Results of bacteriological duplicates are evaluated by calculating the logarithm of each result and determining the range of each pair. Measurement performance specifications are used to determine the acceptability of duplicate analyses. The specifications for bacteriological duplicates in Table A7.1 apply to samples with concentrations >10 cfu/100 mL.

**Method blank**

A method blank is a sample of matrix similar to the batch of associated samples that is free from analytes of interest and is processed simultaneously with and under the same conditions as the samples through all steps of the analytical procedures, and in which no target analytes or interferences are present at concentrations that impact the analytical results for sample analyses. The method blank is carried through the complete sample preparation and analytical procedure. The method blank is used to document contamination from the analytical process. The analysis of method blanks should yield values less than the LOQ. For very high-level analyses, the blank value should be less than 5% of the lowest value of the batch, or corrective action will be implemented.

**Laboratory Blanks**

Laboratory blanks consist of 100 mL aliquots of sterile DI water that are processed in the same manner as a field sample, at the beginning and the end of a sample set. They are used to assess the sterilization techniques employed throughout the sample process. Laboratory blanks will be included at the beginning and the end of the sample set for each sampling event. The analysis of laboratory blanks should yield a value of no colonies detected.

**Positive Control**

The Lab will maintain live *E. coli* in tryptic soy broth and kept refrigerated until needed. Each time a set of samples is run a positive control will be performed in the lab using the same media and 1 mL of live *E. coli* which will be added to 99 mL of sterile DI water that will be run through the filter funnel system and the filter placed on the media. This control should always be positive for *E. coli* after recommended incubation time.

***E. coli* Source Assessment**

**Laboratory Duplicates**

A laboratory duplicate is prepared by taking aliquots of a sample from the same container under laboratory conditions and processed and analyzed independently. A LCSD is prepared in the laboratory by splitting aliquots of an LCS. Both samples are carried through the entire

preparation and analytical process. LCSDs are used to assess precision and are performed at a rate of one per preparation batch. Measurement performance specifications are used to determine the acceptability of duplicate analyses as specified in Table A7.1.

**Method blank**
A method blank is a sample of matrix similar to the batch of associated samples (when available) that is free from the analytes of interest and is processed simultaneously with and under the same conditions as the samples through all steps of the analytical procedures, and in which no target analytes or interferences are present at concentrations that impact the analytical results for sample analyses. The method blanks are performed at a rate of once per preparation batch. The method blank is used to document contamination from the analytical process. For each of the analytical methods used in this project, method blanks should test negative for the target analytes/markers. In addition, no template negative controls will be analyzed for each batch of PCR. Samples associated with a contaminated blank shall be evaluated as to the best corrective action for the samples (e.g. reprocessing or data qualifying codes). In all cases the corrective action must be documented.

Table A7.1 lists the required accuracy, precision, and completeness limits for the parameters of interest. It is the responsibility of the Project Leader to verify that the data are representative. The Project Leader also has the responsibility of determining that the 90 percent completeness criteria is met, or will justify acceptance of a lesser percentage. All incidents requiring corrective action will be documented through use of CARs (Appendix A). Laboratory audits, sampling site audits, and quality assurance of field sampling methods will be conducted by the TSSWCB QAO or their designee at least once per the life of the project.

**Positive Controls**
Positive controls will consist of a laboratory control strain of *E. coli* (QC101) for ERIC-PCR and RiboPrinting and will be included in every preparation batch. For PCR-based library independent BST methods positive controls will consist of fecal DNA or *E. coli* isolate DNA from known human and animal sources and will be included with every preparation batch. Positive controls should always test positive. Samples associated with a failed positive control shall be evaluated as to the best corrective action for the samples (e.g. reprocessing or data qualifying codes). In all cases the corrective action must be documented. The analytical methods are listed in Table A7.1 of Section A7. No EPA-approved methods exist for BST. Detailed SOPs for these methods are provided in Appendix C.

**Field blank**
For each storm event, deionized water will be placed in a clean 5-gallon ISCO® bottle and then processed as a field blank. A field blank is a sample of analyte-free media which has been used to rinse common sampling equipment to check the effectiveness of decontamination procedures. It is collected in the same type of container as the environmental sample, preserved in the same manner and analyzed for the same parameter. The analysis of field blanks should yield values lower than the LOQ. When target analyte concentrations are very high, blank values must be less then 5% of the lowest value of the batch or corrective action will be implemented.

**Failures in Quality Control and Corrective Action**

Notations of blank contamination will be noted in the QPR. Corrective action will involve identification of the possible cause (where possible) of the contamination failure. Any failure that has potential to compromise data validity will invalidate data, and the sampling event should be repeated. The resolution of the situation will be reported to the TSSWCB in the QPR. CARs will be maintained by the TWRI Project Lead.

# Section B6: Equipment Testing, Inspection, & Maintenance Requirements

To minimize downtime of all measurement systems, spare parts for laboratory equipment (Table B6.1) will be kept in the laboratory (when feasible), and all laboratory equipment will be maintained in working condition. Equipment will be tested, maintained, and inspected in accordance with manufacturer's instructions and recommendation in Standard Methods for the Examination of Water and Wastewater, 22nd Edition. Maintenance and inspection logs will be kept on each piece of laboratory equipment. Records of all tests, inspections, and maintenance will be maintained and log sheets kept showing time, date, and analyst signature. These records will be available for inspection by the TSSWCB. Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the QPR. CARs will be maintained by the TWRI Project Lead.

**Table B6.1 Equipment Inspection and Maintenance Requirements**

| Equipment | Relevant Testing, Inspection & Maintenance Requirements |
|---|---|
| Thermometers | SM 9020 B 3.a |
| PCR Thermal cycler | Per manufacturer |
| RiboPrinter | Per manufacturer & annual preventative maintenance |
| Water deionization units | SM 9020 B 3.d |
| Media dispensing apparatus | SM 9020 B 3.f |
| Autoclaves | SM 9020 B 3.h |
| Refrigerator | SM 9020 B 3.i |
| Ultra Low Freezer | SM 9020 B 3.j |
| Membrane filter equipment | SM 9020 B 3.k |
| Ultraviolet sterilization lamps | SM 9020 B 3.l |
| Biological safety cabinet | SM 9020 B 3.m |
| Incubators | SM 9020 B 3.o |
| Glassware and plastic ware | SM 9020 B 3.a |
| Utensils and containers | SM 9020 B 3.b |
| Dilution water bottles | SM 9020 B 3.c |
| pH probe | per manufacturer |
| Dissolved Oxygen sensor | per manufacturer |
| temperature probe | per manufacturer |
| Hanna HI 83414 Optical Turbidity Sensor | per manufacturer |
| Shimadzu TOC-VCSH & TNM1 | per manufacturer – 2-3 times per year |
| Unity Scientific SmartChem 200 | per manufacturer |
| specific conductance meter | per manufacturer |

# Section B7: Instrument Calibration and Frequency

Each instrument has a specialized procedure for calibration and a specific type of standard used to verify calibration. The instruments requiring calibration are listed below in Table B7.1. All calibration procedures will meet the requirements specified in the USEPA-approved methods of analysis. The frequency of calibration as well as specific instructions applicable to the analytical methods recommended by the equipment manufacturer will be followed. All information concerning calibration will be recorded in a calibration logbook by the person performing the calibration and will be accessible for verification during either a laboratory or field audit.

All instruments or devices used in obtaining environmental data will be used according to appropriate laboratory or field practices. Written copies of SOPs are available for review upon request.

Standards used for instrument or method calibrations shall be of known purity and be National Institute of Standards and Technology (NIST) traceable whenever possible. When NIST traceability is not available, standards shall be of American Chemical Society or reagent grade quality, or of the best attainable grade. All certified standards will be maintained traceable with certificates on file in the laboratory. Dilutions from all standards will be recorded in the standards log book and given unique identification numbers. The date, analyst initials, stock sources with lot number and manufacturer, and how dilutions were prepared will also be recorded in the standards log book.

Failures in any testing, inspections, or calibration of equipment will result in a CAR and resolution of the situation will be reported to the TSSWCB in the QPR. CARs will be maintained by the TWRI Project Lead.

**Table B7.1 Instrument Calibration Requirements**

| Equipment | Relevant Calibration Requirement |
|---|---|
| RiboPrinter | Per manufacturer & annual preventative maintenance |
| PCR Thermal Cycler | Per manufacturer |
| Shimadzu TOC-VCSH & TNM1 | Per manufacturer |
| Unity Scientific SmartChem 200 | Per manufacturer |
| Hanna HI 83414 Optical Turbidity Sensor | per manufacturer |
| specific conductance meter | per manufacturer |

# Section B8: Inspection/Acceptance Requirements for Supplies and Consumables

All standards, reagents, media, plates, filters, and other consumable supplies are purchased from manufacturers with performance guarantees, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality, initialed by staff member and marked with receipt date. Volumetric glassware is inspected to ensure class "A" classification, where required. Media will be checked as described in quality control procedures. All supplies will be stored as per manufacturer labeling and discarded past expiration date. In general, supplies for microbiological analysis are received pre-sterilized, used as received, and not re-used.

# Section B9: Data Acquisition Requirements (Non-direct Measurements)

All required data to be used for this project will be collected in accordance with this QAPP.

***BST Analysis***

Data analyzed using BST analysis methods for this project will consist of data produced during the course of this study under the specifics of this QAPP, or generated under previous TSSWCB studies with accepted QAPPs.

BST results from project specific samples will be compared to known source *E. coli* DNA isolated housed in the Texas *E. coli* BST Library. Each of these isolates was collected and processed in the same manner as known source samples collected and processed through this project.

# Section B10: Data Management

**Laboratory Data**

All field samples (known-source fecal samples, surface water runoff samples, and soil samples) will be logged upon receipt, COC forms (if applicable) will be checked for number of samples, proper and exact identification number, signatures, dates, and type of analysis specified. TSSWCB will be notified if any discrepancy is found and laboratory analysis will not occur until proper corrections are made. All samples will be stored at 4ºC until analysis. Bacteriological samples will be given a unique identification number and logged into a Microsoft Excel database used to store field data. All backup and safety features of this database are the same as explained above. Enumerated bacteriological data will be manually entered into the database system for electronic storage. At least 10% of all data manually entered in the database will be reviewed for accuracy by the Project Lead to ensure that there are no transcription errors. Hard copies of data will be printed and housed at the generating laboratory for a period of five years. Any COC's and bacteriological records related to QA/QC of bacteriological procedures will be housed at SAML.

**Data Validation**

Following review of laboratory data, any data that is not representative of environmental conditions, because it was generated through poor field or laboratory practices, will not be submitted to the TSSWCB. This determination will be made by the SAML Project Co-Lead, NAWA Project Co-Lead, TWRI QAO, TSSWCB QAO, and other personnel having direct experience with the data collection effort. This coordination is essential for the identification of valid data and the proper evaluation of that data. The validation will include the checks specified in Table D2.1.

**Data Reporting**

Data will be reported in the project final report and peer reviewed publications that are developed form project findings.

**Data Dissemination**

At the project's conclusion, the TWRI Project Lead will provide a copy of the complete project electronic database via recordable CD-ROM media to the TSSWCB PM, along with the final report. TSSWCB may elect to take possession of all project records. These records will be maintained according to the Project Records retention schedule in Table A.9. Summaries of the data will be presented in the final project report. TSSWCB may disseminate the project final report.

# Section C1: Assessments and Response Actions

Table C1.1 presents the types of assessments and response action for activities applicable to this QAPP.

**Table C1.1: Assessments and Response Actions**

| Assessment Activity | Approximate Schedule | Responsible Party | Scope | Response Requirements |
|---|---|---|---|---|
| Status Monitoring Oversight, etc. | Continuous | TWRI | Monitor project status and records to ensure requirements are being fulfilled. Monitoring & review performance & data quality | Report to TSSWCB in QPR. |
| Equipment testing | As needed | SAML/BAEN/ NAWA | Pass/Fail equipment testing | Repair or replace |
| Data completeness | As needed | SAML/BAEN/ NAWA/TWRI | Assess samples analyzed vs. planned analysis | Reanalyze or amend objectives |
| Laboratory Inspections | TBD by TSSWCB | TSSWCB | Analytical and QC procedures in the laboratory | 30 days to respond to TSSWCB with corrective actions |
| Technical systems audit | As needed | TSSWCB | Assess compliance with QAPP; review facility and data management as they relate to the project | 30 days to respond to TSSWCB with corrective actions |
| Monitoring Systems Audit | Once per life of project | TSSWCB | Assess compliance with QAPP; review field sampling and data management as they relate to the project | 30 days to respond to TSSWCB with corrective actions |

**Corrective Action**

The Project Leaders are responsible for implementing and tracking corrective action procedures as a result of audit findings. Records of audit findings and corrective actions are maintained by the TSSWCB QAO.

If audit findings and corrective actions cannot be resolved, then the authority and responsibility for terminating work is specified in agreements or contracts between participating organizations.

# Section C2: Reports to Management

QPRs will be generated by TWRI and will note activities conducted in connection with the water quality monitoring program, items or areas identified as potential problems, and any variation or supplement to the QAPP. CARs will be utilized when necessary (Appendix A) and will be maintained in an accessible location for reference. CARs that result in changes or variations from the QAPP will be made known to pertinent project personnel, documented in an update or amendment to the QAPP and distributed to personnel listed in Section A3.

TWRI will work with BAEN, NAWA, SAML and USDA-ARS to develop a Final Report for submission to the TSSWCB that summarizes activities completed, conclusions reached during the project, and the extent to which project goals and measures of success have been achieved.

# Section D1: Data Review, Validation, and Verification

All data obtained from field and laboratory measurements will be reviewed and verified for integrity, continuity, reasonableness, and conformance to project requirements, and then validated against the DQOs outlined in Section A7. Only those data that are supported by appropriate QC data and meet the DQOs defined for this project will be considered acceptable for use.

The procedures for verification and validation of data are described in Section D2, below. Project Leaders are responsible for ensuring that field and laboratory data collected are properly reviewed, verified, and submitted in the required format for the project database. TWRI is responsible for validating that all data collected meet the DQOs of the project are suitable for submission to TSSWCB.

# Section D2: Validation and Verification Methods

All data will be verified to ensure they are representative of the samples analyzed and locations where measurements were made, and that the data and associated QC data conform to project specifications. The TWRI Project Lead is responsible for the integrity, validation, and verification of the data each field and laboratory task generates or handles throughout each process. The field and laboratory QA tasks ensure the verification of field data, electronically generated data, and data on COC forms and hard copy output from instruments.

Verification, validation, and integrity review of data will be performed using self-assessments and peer review, as appropriate to the project task, followed by technical review by the manager of the task. The data to be verified (listed by task in Table D2.1) are evaluated against project specifications (Section A7 and Section B5) and are checked to ensure the verification of raw data for errors, especially errors in transcription, calculations, and data input. Potential outliers are identified by examination for unreasonable data, or identified using computer-based statistical software. If a question arises or an error or potential outlier is identified, the manager of the task responsible for generating the data is contacted to resolve the issue. Issues that can be corrected are corrected and documented electronically or by initialing and dating the associated paperwork. If an issue cannot be corrected, the task manager consults with the TSSWCB QAO to establish the appropriate course of action, or the data associated with the issue are rejected. Performance of these tasks is documented by completion of the data review checklist (Appendix D).

Project Leaders and TWRI are responsible for validating that the verified data are scientifically sound, defensible, of known precision, accuracy, integrity, meet the DQOs of the project, and are reportable to the TSSWCB.

**Table D2.1: Data Review, Verification, and Validation Procedures**

| Data to be Verified | Field† Supervisor | Laboratory Supervisor | PM/QAO Task‡ |
|---|---|---|---|
| Collection & analysis techniques consistent with SOPs & QAPP | X | X | X |
| Field QC samples collected for all parameters as prescribed in the QAPP | X | | X |
| Field documentation complete | X | | X |
| Instrument calibration data complete | X | X | X |
| Sample documentation complete | X | X | X |
| Field QC results within acceptance limits | X | | X |
| Sample identifications | X | X | X |
| Chain of custody complete/acceptable | X | X | X |
| Sample preservation and handling | X | X | X |
| Holding times | X | X | X |
| Instrument calibration data | X | X | X |
| QC samples analyzed at required frequencies | | X | X |
| QC samples within acceptance limits | | X | X |
| Instrument readings/printouts | X | X | X |
| Calculations | X | X | X |
| Laboratory data verification for integrity, precision, accuracy, and validation | | X | X |
| Laboratory data reports | | X | X |
| Data entered in required format | X | X | X |
| Site ID number assigned | X | | X |
| Absence of transcription error | X | X | X |
| Reasonableness of data | X | X | X |
| Electronic submittal errors | X | X | X |
| Sampling and analytical data gaps | X | X | X |

† Field and Laboratory Supervisor may be the same person

‡ TSSWCB PM / QAO will monitor data for QA/QC purposes as needed.
All other entities are required to inspect 100% of the data prior to approval

# Section D3: Reconciliation with User Requirements

Data produced by this project will be evaluated against the established DQOs and user requirements to determine if any reconciliation is needed. Reconciliation concerning the quality, quantity or usability of the data will be reconciled with the user during the data acceptance process. Corrective Action Reports will be initiated in cases where invalid or incorrect data have been detected. Data that have been reviewed, verified, and validated will be summarized for their ability to meet the data quality objectives of the project and the informational needs of water quality agency decision-makers and watershed stakeholders.

The final data for the project will be reviewed to ensure that it meets the requirements as described in this QAPP. Data summaries along with descriptions of any limitations on data use will be included in the final report. Only data that has met the data quality objectives described in this QAPP will be reported and included in the final project report. Since BST is an evolving science and no EPA-approved protocols currently exist, a discussion of the uncertainties surrounding source identification and the appropriate use of BST results will be included in the project final report. Data and information produced through this project will provide needed information pertaining to Texas BST efforts.

# References

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# APPENDIX A

## Corrective Action Report

**Corrective Action Report**
**CAR #:_____**

Date:_____ Area/Location:_____

Reported by:_____ Activity:_____

State the nature of the problem, nonconformance, or out-of-control situation:
_____
_____
_____
_____
_____

Possible causes:
_____
_____
_____

Recommended corrective action:
_____
_____
_____

CAR routed to:_____
Received by:_____

Corrective Actions taken:
_____
_____
_____
_____

Has problem been corrected?: YES NO

Immediate Supervisor:_____

Project Leader:_____

Quality Assurance Officer:_____

# APPENDIX B

## Chain of Custody Record
## &
## Sheets of Lading for Fecal Specimen Transport Template

# CHAIN OF CUSTODY RECORD

| **Project:** | **Remarks:** |
|---|---|
| **Name and signature of collector:** | **Air bill #** |

| **Station ID** | **Sample ID** | **Media Code** | **Sample Type** | **Preservative** | **Collection Date** | **Time** |
|---|---|---|---|---|---|---|
| | | | | | | |
| | | | | | | |
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| Relinquished by: | Date: | Time: | Received by: | Date: | Time: |
|---|---|---|---|---|---|
| Laboratory Notes: | | | | | |
| Media Code: (**FS**) Fecal Sample; (**SS**) Sewage Sample | | | | | |

# Sheets of Lading for Fecal Specimen Transport

**(Collector's Organization)**

FY14-15 Bacteria Growth, Persistence, and Source Assessment in Rural Texas Landscapes and Streams

(Collector's Name and title)
(Collector's Phone Number)

**In case of EMERGENCY:**
(Contact name and number)

**Date:_____Time:_____**

**Sample:** Fecal **Hazard:** Bacteria

**Species/ Animal**:_____

**Photo: Yes No**

**GPS (or other location note)**: Lat_____Long_____

_____

**Other Info:_____**

**Technician:_____**

# APPENDIX C

## BST STANDARD OPERATING PROCEDURES

## C-1: Collection of Fecal Samples for Bacterial Source Tracking

1. Only fresh fecal samples of known origin should be collected. Specifically, fecal samples should be obtained in one of five ways:
   a. Collected from animals visually observed defecating by technician.
   b. Collected from trapped animals.
   c. Collected from intestines of animals legally harvested.
   d. Collected from the intestines of animals recently killed by cars (within 24 hours).
   e. Human (wastewater) samples collected from individual septic tanks, composite septic samples from pump trucks, from wastewater treatment plant influent (for plants with secondary disinfection or lagoon treatment), or from lagoon treatment effluents.

2. Samples should be carefully collected to avoid contamination. Samples on the ground should be collected with a sterile spatula, or similar device, while avoiding collection of material in contact with soil or other possible sources of contamination. Intestinal samples should be collected from animals by using sterile loops inserted anally or by cutting into the intestine using a sterile scalpel. Wastewater samples can initially be collected with sterile bottles, or other suitable device and then transferred to the fecal tubes described below.

3. Each fecal sample should be placed in a new, sterile fecal tube (Sarstedt, cat# 80.734.311). Tubes should be filled approximately ¾ full (can provide less material for smaller animals)

4. Samples should be refrigerated (~4°C) or kept on ice following collection.

5. At the time of sampling, record detailed information on the tube regarding the sample including:
   a. Sampling date
   b. Sampling time
   c. Animal species
   d. Sample location (e.g., GPS coordinates [preferred] or town, city, and/or county)
   e. Sample collector's name/initials
   f. Any other pertinent information, e.g. sex of animal or any other easily obtainable information such as beef cattle versus dairy cattle

6. Notify the appropriate lab via email or phone as soon as possible (prior to or immediately following sample collection) with an estimated number of samples that will be shipped and the expected date of shipment. This will allow lab to make appropriate preparations to process the samples immediately upon arrival.

SAML
Emily Martin or Heidi Mjelde
emartin@ag.tamu.edu or
hmjelde@ag.tamu.edu
979-845-5604

7. Samples should be shipped (at 4ºC) as soon as possible (within **3 days**) to the appropriate lab (address below). 'Blue-ice' or freezer blocks should be used to keep the samples cool, but not frozen during transport. Samples should be placed in secondary containment such as large Whirl-Pak or zip-top bags.

8. Notification of shipment should be sent to the appropriate lab via email or phone (see contact info above) no later than the day of overnight shipping. Notification should include tracking number and direct collections contact person for confirmation upon receipt of samples.

9. Ship samples (and COCs) in insulated coolers (marked on outside to indicate that contents are perishable) with sufficient ice packs to maintain ~4°C to:

SAML
Terry Gentry
Texas A&M University
Soil & Crop Sciences; Heep Center 539
370 Olsen Blvd
College Station, TX 77843
979-845-5604

## C-2: Laboratory Protocol for Isolation and Confirmation of *Escherichia coli* from Fecal Specimens

*Note: All collection and handling of fecal specimens should be performed using protective gear (e.g. latex or nitrile gloves). Specimens should be handled aseptically to ensure sample quality and minimize exposure of personnel to pathogens. All feces collected will be placed in screw capped sterile containers. Containers will be labeled with: Name of collector, date, species, GPS location, and photo of specimen before collection. Containers will then be placed in ziplock biohazard bags with lading pouch. Information will be written on lading report and placed in the bag. Fecal specimens will be placed in an insulated cooler on ice during transport.*

*Note: All handling of fecal specimens and cultures will be performed using a Class 2 biological safety cabinet to minimize the exposure of laboratory personnel to pathogens.*

1. Using a bacteriological loop, streak a loop full of fecal material onto a labeled modified mTEC agar plate (USEPA-821-R-02-023, Modified USEPA Method 1603; http://www.epa.gov/nerlcwww/1603sp02.pdf) for isolation of *E. coli* colonies.

2. Incubate the plate inverted at 44.5±0.2°C for 20 to 24 h.

3. Examine the plate for presumptive *E. coli* colonies, which will appear red or magenta colored.

4. Select up to three presumptive *E. coli* colonies and streak each colony for purity onto a labeled nutrient agar MUG (NA-MUG) plate.

5. Invert and incubate plates at 35 to 37°C for 20 to 24 h.

6. Examine the cultures using a **long-wave handheld UV lamp**. If there is a mixture of fluorescent and non-fluorescent colonies, select a well isolated fluorescent colony and streak again onto NA-MUG for purity.

7. At the discretion of the laboratory, additional biochemical tests such as urease, indole, and citrate tests may be performed.

## C-3: Archival of *Escherichia coli* Isolates

*Note: All handling of cultures will be performed using a Class 2 biological safety cabinet to minimize the exposure of laboratory personnel to pathogens. These isolates should be from colonies which have been plated for purity several times and lab personnel are confident purity has been achieved.*

1. Select a well-isolated colony of purified *E. coli*. (Examine the cultures using a long-wave handheld UV lamp, colonies will fluoresce).

2. Using a bacteriological loop, transfer the colony to a labeled sterile cryovial containing 1 mL of tryptone soy broth (TSB) with 20% reagent grade glycerol.

3. Firmly cap the cryovial and verify that the cells have been resuspended by vortexing for several seconds; then plunge into liquid nitrogen until frozen.

4. Immediately transfer to a cryostorage box and place in -70 to -80°C freezer. Cultures may be stored for several years under these conditions.

5. To recover cultures from frozen storage, remove the cultures from the freezer and place the cryovials in a freezer block. *Do not allow cultures to thaw.*

    a. Using a bacteriological loop, scrape the topmost portion of the culture and transfer to growth medium, being careful not to contaminate the top or inside of the vial. Invert and incubate plates at 35 to 37°C for 20 to 24 h.

    b. Reclose the cryovial before the contents thaw and return to the freezer.

## C-4: ERIC-PCR of *Escherichia coli*

1. Select isolated colonies from overnight cultures of *E. coli* isolates on Brain-Heart Infusion (BHI) plates.

2. Transfer colonies using a 1 µL loop to a sterile microfuge tube containing 100 µL of sterile molecular grade water; vortex briefly to suspend cells.

3. Prepare sufficient PCR Master Mix for samples, including one blank per 10 samples to account for volume loss due to repeat pipetting. Prepare Master Mix for each sample as noted below. One full PCR batch on the MJ Research Cycler 48 well-plate will have 46 samples, *E. coli* QC101, and a no template control.

**ERIC-PCR Master Mix – 24 samples + 2 blanks, prepare X 2 for full 48-well plate**

| MASTER MIX | Amt (uL) | Final Calc | Final Units |
|---|---|---|---|
| dH2O | **819** | | |
| 10X PCR buffer I w Mg | **130** | 1 | X (1.5 mM) |
| 20 mM dNTP | **13** | 200 | uM each |
| ERIC Primer Mix | **130** | 600 | nM each |
| BSA (30 mg/ml) | **65** | 1.5 | ug/uL |
| AmpliTaqGold (Units) | **13** | 2.5 | Units/rxn |

4. Dispense 45 µl of Master Mix for each sample into the appropriate well of PCR plate.

5. Briefly vortex cell suspensions, then add 5 µl of each cell suspension to the appropriate PCR well.

6. Carefully seal plate using an adhesive PCR cover.

7. Load the plate into the thermal cycler and run under the "ERIC-PCR" program with the following cycling conditions:

    a. Initial denaturation at 95°C for 10 min

    b. 35 Cycles:
        i. Denaturation at 94°C for 30 sec
        ii. Annealing at 52°C for 1 min
        iii. Extension at 72°C for 5 min

    c. Final Extension at 72°C for 10 min

8. Store completed reactions at -20°C until analyzed by gel electrophoresis.

9. Prepare a 250 mL, 2% agarose gel using a 500 mL bottle. Add 250 mL of 1 X Tris/Borate/EDTA (TBE) buffer and 5.0 g agarose. Microwave until agarose is fully dissolved, tighten cap and let cool 1 to 2 minutes, then pour agarose into casting tray with 30-tooth, 1 mm thick comb.

10. Allow gel to solidify for approximately 30 minutes on the bench, then without removing comb place in Ziploc bag and solidify overnight in the refrigerator. The next day carefully remove comb, transfer to gel tank in cold room (4°C) containing pre-cooled 1X TBE buffer. Replace TBE in gel tank after it has been used twice.

11. The following items will be needed for electrophoresis:

    100 bp ladder (0.33 µg/10 µL) (1500 µL final, enough for 150 lanes)

    200 µL Roche DNA Marker XIV (Cat. #1721933) 0.25 µg/µL 100 bp ladder (add reagents below to a full tube of marker)

    300 µL 6X ERIC-PCR loading buffer (see recipe below)

    150 µL 10X PCR buffer

    850 µL molecular grade water

    Store in cold room

    6X ERIC-PCR Loading Buffer

    25 mg bromphenol blue (0.25%)

    1.5 g ficoll 400 (15%)

    Add molecular grade water to 10 mL, divide into 1 mL aliquots and freeze, the aliquot currently being used can be stored in the cold room

    ERIC-PCR Blank

    100 µL 10X PCR buffer

    200 µL 6X ERIC-PCR loading buffer

    900 µL molecular grade water

    Store in cold room

    Ethidium Bromide Stain (0.5 µg/mL)

1250 mL 1X TBE

62.5 µL ethidium bromide (Sigma, 10 mg/mL)

Store covered at room temp, can use up to 5 times by adding 10 µL ethidium bromide each additional use

12. Mix 10 µL of 6X ERIC-PCR Loading Buffer to each PCR well and mix with pipette tip.

13. Load the gel in the cold room as follows (max. of 23 samples + QC101 + NTC per gel):

    a. Load 10 µl of 100 bp ladder (0.33 µg) into the first lane

    b. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

    c. Load 10 µl of 100 bp ladder (0.33 µg)

    d. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

    e. Load 10 µl of 100 bp ladder (0.33 µg)

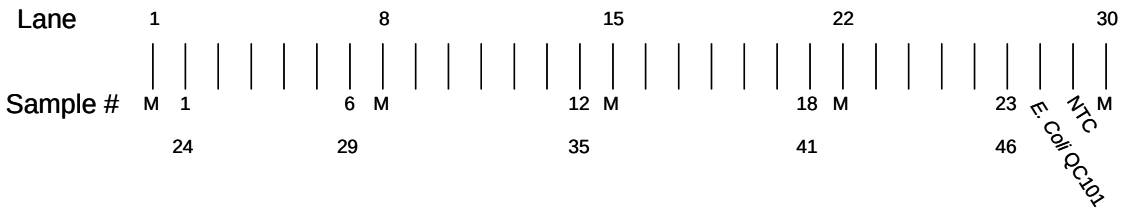
    f. Load 10 µl of sample ERIC-PCR reactions into next 6 lanes

    g. Load 10 µl of 100 bp ladder (0.33 µg)

    h. Load 10 µl of sample ERIC-PCR reactions into next 5 lanes

    i. Load PCR Batch *E. coli* QC101 and NTC into next 2 lanes

    j. Load 10 µl of 100 bp ladder (0.33 µg)




If running a gel with fewer samples, follow steps above until last sample, followed by *E. coli* QC101, NTC and ladder, then load ERIC-PCR Blank into remaining lanes on gel.

14. Start electrophoresis power supply set at 100 volts, run for 1 hour.

15. Stop power supply, set time to “000”, set voltage to 200 and start circulating pump at setting #2, run for 4 hours.

16. After electrophoresis, stain gel in Ethidium Bromide Stain for 20 minutes with agitation (save stain, see Step 13).

17. Destain gel for 10 minutes in 1X TBE buffer. Save destain, can be used 3 times then discard.

18. Follow Gel Logic 200 SOP for image capture. Save digital photograph as a TIFF file (default) and print a hardcopy for notebook.

# C-5: RiboPrinting of *Escherichia coli*

## Storing and Handling Disposables

Check the lot expiration date on each label for details and rotate the stock to optimize use.

Heating membrane and probe (MP) Base

After storage and the temperature changes that occur during shipment, the oxygen in the buffer loaded in the MP base may need to be removed before use. This is called degassing and is accomplished by heating the base pack overnight in your incubator.

To degas buffer:

1. Place enough MP base packs for the next day's production in their storage pouches in an incubator set at 37°C.

2. Allow the base pack to degas for 16 to 24 hours prior to loading in the characterization unit. You may do this while you are incubating samples, since the base packs are sealed in their pouches. This procedure allows you to start a batch immediately at the beginning of the next shift.

3. If you do not use the heated base packs, you can return them to storage and reuse them. These base packs should be heated again before reuse since temperature cycling affects oxygen content in the buffer.

Preparing Lysing Agent (for *Staphylococcus* and lactic-acid bacteria only)

Lysing agent (A and B) is shipped frozen and must be stored at -20°C. Lysing agent must be thawed before use. This only takes about 5 minutes. If the lysing agent will not be used again for more than 2 hours, the material should be returned to the freezer. Lysing agent can be re-frozen several times with no effect on performance.

**Sample Preparation Procedures**

**Incubate and Inspect the Samples**

Use BHI agar plates prepared within the last 30 days. Do not use plates that appear dry or dehydrated. Such plates can cause problems when you attempt to "pick" the colonies for use in the RiboPrinter® system.

1. Locate the 250 mL twist-top bottle of sample buffer supplied in Pack # 1. Install the twist cap.

2. Transfer about 5 mL of buffer to a sterilized disposable 15 mL intermediate working tube.

3. Add sample buffer to microcentrifuge tubes

    1. Place a sterile 0.65 mL microfuge tube in each of the eight holes in the lower row of the sample preparation rack.

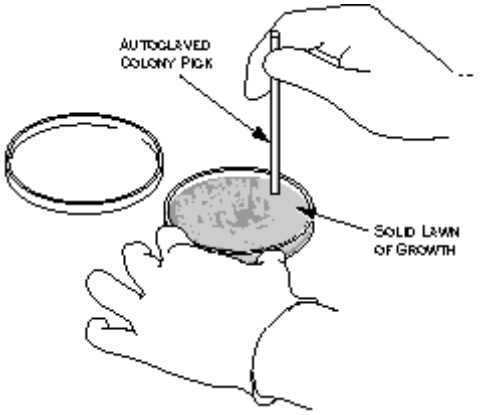
    2. For Gram negative samples (including *E. coli*), add 200 µL of sample buffer from the intermediate tube.

       For Gram positive samples (e.g. *S. aureus* and *L. innocua* QC strains), add 40 µL of sample buffer.

    3. Close the lids on the tubes.

**Harvest the Samples**

1. Using autoclaved colony picks and making certain not to gouge the agar, carefully place the pick into one of the single colonies or the lawn. You need a sample area at least equal to that of the bottom of the colony pick. In most cases you will need to harvest from the lawn area of the plate. If you are working with large colonies, a single colony will be adequate.




2. <u>For Gram negative samples (e.g. *E. coli*), perform 1 pick placed into 200 µL of sample buffer.</u>

CAUTION! Do not try to use the same pick twice on a plate. You need to harvest only enough sample to cover the bottom surface of the pick. Make sure the end of the pick is flat, if not, use a different pick.

CAUTION! Do not overload the harvesting pick. Collect only enough sample to cover the base of the pick. Over sampling will cause inaccurate results. Over sampling is a particular problem with *Staphylococcus*.

**Mix the Samples**

***WARNING!*** *Perform sample preparation using a Class 2 biological safety cabinet since aerosols may be formed during mixing of the samples.*

1. Making certain not to touch the sample end of the pick, place the pick into one of the filled sample tubes.

2. While holding the tube with the open end facing away from you, carefully attach the pick to the hand-held mixer. The fit of the pick in the coupling will be loose.

*WARNING! Do not turn on the mixer unless the pick is inside the sample tube and below the surface of the liquid. Turning the unit on at other times will cause the sample to aerosolize and may cause contamination.*

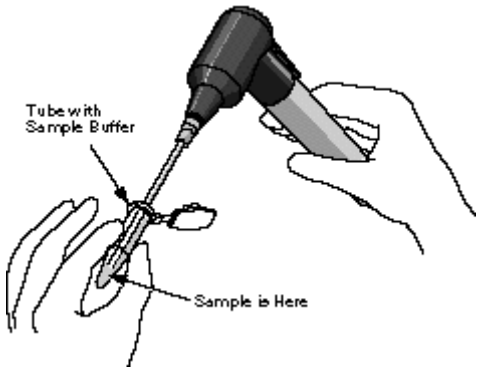
3. Press the ON lever on the mixer for about 5 seconds.

4. Release the lever and carefully remove the colony pick. The sample liquid should appear turbid.

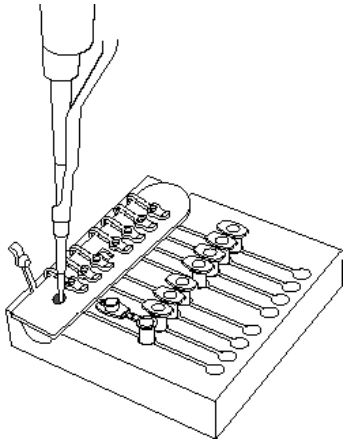
5. For **Gram positive samples only**, (e.g. *Staphylococcus* and *Listeria*) locate a new colony pick and repeat the steps for harvesting and mixing samples, adding a second sample to the original tube. Discard the used picks in a biowaste bag.

6. Cap the sample tube.

7. Move the tube to the top row of the sample preparation rack. This indicates that the tube is filled.



**Transfer the Samples to the Sample Carrier**



1. Open the lid covering the first well of the sample carrier.

2. Using a 100 µL pipetter, pipette 30 µL of sample from the microcentrifuge tube into the well.

3. Close the lid cover for the well.

4. Repeat for remaining samples using a new pipet tip for each sample.

*CAUTION! Transfer the sample carrier to the Heat Treatment Station within 2 hours. If you wait longer than 2 hours, you will have to discard the sample carrier and begin again for this batch.*

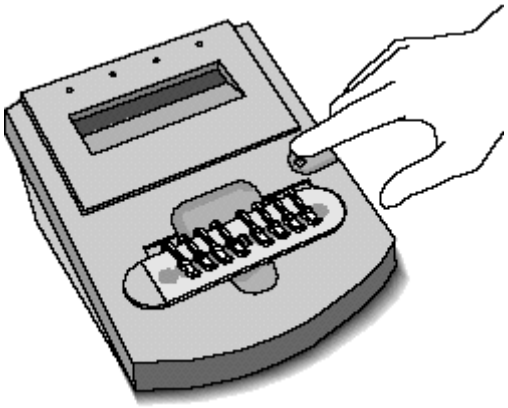
5. Lightly wipe down the outer surfaces of the sample carrier with a lab wipe wetted with surface disinfectant (10% bleach or 70% alcohol).

6. Write down the name or code you use to identify the sample and the well number in the sample carrier for each sample using a sample log sheet.

**Place the Sample Carrier in the Heat Treatment Station and Process the Sample Carrier**

1. Place the sample carrier into the Heat Treatment Station. The display on the Heat Treatment Station will show **Insert**, if power is available. If the display is blank, make certain that the power cord on the back of the station is properly connected.

After you insert the carrier, the display shows **Press Button.**



2. Press the button on the Heat Treatment Station.

   The display shows **Warm up** and counts down from **10** while the station is warming up. The actual warm up cycle varies with the condition of the room and the heat treatment station. Normal time is about 4 minutes.

   When the station reaches operating temperature, the display changes to **Heat** and counts down from **13.** This represents each minute of heat treatment.

   The indicator message changes to **Cool.** The display counts down from **9**, indicating the minutes remaining in the cooling cycle**.** If necessary, you can remove the carrier as soon as the **Cool** message appears.

3. The heat treatment step is finished when the display shows **READY** and counts down from **90.** The display will flash and an audible beep will sound three times. The alarm will then beep once every 10 minutes until the sample is removed or 90 minutes elapses.

*Caution! The heat-treated samples must be used within the 90-minute period at room temperature or they must be discarded. The heat-treated samples may be stored at this point (prior to adding Lysis Agents, if required) for 1 week at 4 °C, or for several months at -70 °C.*

**Add the Lysing Agents (for *Staphylococcus* and lactic-acid bacteria only)**

1. Using a 10-µL pipetter and new tips for each addition, add 5 µL of Lysing Agents A and B to each sample. Note: this step is omitted for *E. coli* as it has no effect on ribopatterns. Lysing Agents were specifically developed for *Staphylococcus* and Lactic-Acid bacteria samples.

*Caution! This step must be performed just prior (within 10 minutes) of loading the samples into the RiboPrinter and starting the run.*

**Creating and Loading a Batch**

There are three options under the Operations menu for creating standard batches:

- *Eco*RI batches (VCA)
- *Pst*I batches (VCB)
- *Pvu*II batches (VCC)

You can also create special batches:

- Restriction Enzyme Flexibility batches
- **Substitute Enzyme batches (including *Hin*d III)**

From the Instrument Control Base Window:

1. Move the pointer to Operations and click with the mouse button. The Operations menu appears.

2. Move the pointer to Create Substitute Enzyme Batch and click with the mouse button.

3. Use the View menu to remove any optional items you do not wish to fill in. The system requires at least Sample Type and RiboGroup Library information for each sample. You cannot remove these options. The **Clear** option de-selects the **Use Default ID Libraries.** You will have to enter a DuPont ID and Custom ID library name for all samples. These become required fields and the system will make you enter data before you can save the information in this window.

*CAUTION! If you change the display after you have entered information, you will lose all the information in the window. The window will redraw with a new blank display showing the items you have selected.*

4. To enter information about the sample, click on the **View** button with the mouse button, and then click on **Sample Items**. Click on the options you want to display.

5. Enter your initials and any comment you want to record about the batch.

6. Select the lot number fields and record for all reagents.

*CAUTION! All fields must be completed or the system will not let you start processing the batch.*

4. For each well in the sample carrier, choose the type (Sample or Control [QC Number]) from the Sample Type field. The system defaults to Sample.

5. Once you define the Sample Type as Sample, type in the name you actually want to use. This information will appear as Sample Label in the Data Analysis software screens.

6. You can change the RiboGroup library name if needed. Do this by clicking on the button next to the field with the mouse button. A pop up menu appears listing your choices. If you want to add a new library name, move the pointer to the line and click with the mouse button to get a cursor, then type in the new library name. Once you have saved this file, the new name will be added to the pop up list for future use. Do **NOT** change the DuPont ID field. If you select one of the QC strains, the system automatically enters QC in the DuPont ID and RiboGroup Library fields. Do not change these names. If you wish, you may enter a name for the Custom ID library.

7. Repeat for the other seven samples.

8. Click on Save and Submit Batch to Instrument.

**Loading Disposables**

Follow the screen prompts to load disposables and check the DNA Prep Waste. The icons on the window will flash red to tell you to remove and load an item. The screen prompts you about which Separation and Transfer chamber to use for the membrane and gel cassette. The LDD Pipette will move to physically block you from placing samples in the wrong chamber.

***CAUTION!*** *Do not try to move the pipette manually. You will cause the system to lose the step count. This can result in the loss of batch data. If the pipette is blocking the S/T chamber that you are instructed to use, STOP. Call Customer Support.*

***CAUTION!*** *Do not load disposables until you are prompted by the system. If you try to load them earlier, the alarm will sound as long as the doors are open. If you do load disposables ahead of time, the MP Base will be moved to the wrong position and you will not be able to begin processing the batch. You will not be able to move the MP base manually.*

**1. Check the DNA Preparation Waste Container**

1. The DNA Prep waste container must be visually checked before every batch. If the container looks nearly full (about 1 inch from the top), remove the container, unscrew the cap and empty into the liquid biohazard waste.

*WARNING! Do not tip the DNA Preparation waste container when you remove it.*

*WARNING! Do not unscrew the cap from the DNA Preparation waste container if the fluid level has risen into the cap. First pour the excess waste liquid into the liquid biohazard waste.*
*WARNING! When replacing container make sure that the cap is properly threaded in place. If the cap is only partially threaded, it can snag the pipette during operation.*

2. Load the Sample Carrier

3. Place the sealed carrier into the labeled slot on the far right of the characterization unit.

4. Push the sample carrier down firmly until it snaps into place.

*CAUTION! Place the rounded edge of the sample carrier on your right as you view the characterization unit. Position the carrier this way to insure correct identification of the sample wells.*

**3. Load the DNA Prep Carrier**

Remove the DNA Prep carrier from the refrigerator.

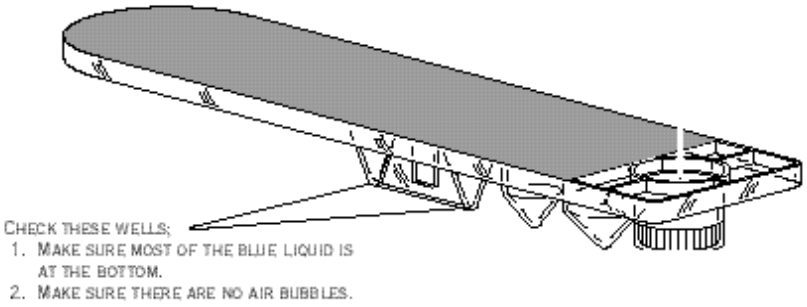
1. Check the wells in the carrier. If most of the liquid appears to be in the bottom of the wells and there are no bubbles, go to step 3. Otherwise **lightly tap the side of the carrier a few times with your finger to release any material that has adhered to the lid.**

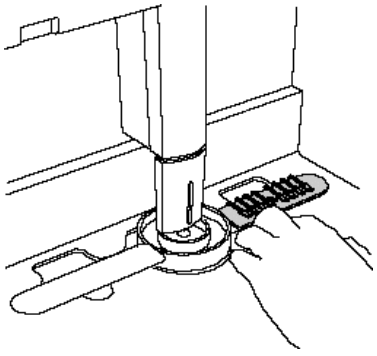
2. CAUTION! Do not tap the carrier briskly. This may cause the marker to degrade which can create inaccurate results.

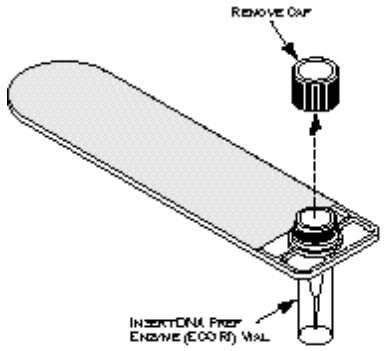
3. Remove a vial of DNA Prep Enzyme (*Hin*d III or *Eco*R I) from the freezer. ***Hin*d III (NEB Cat. #R0104M) is prepared in a Sarstedt 500-µL microfuge tube (Cat. #72730-005) as a 50 U/µL working stock as follows.**

   <u>50 U/µL:</u> 26.5 µL *Hin*d III and 26.5 µL of NEB 10X Buffer 2

   **During addition of the Buffer, mix enzyme and buffer to homogeneity by stirring with the micropipette tip.**



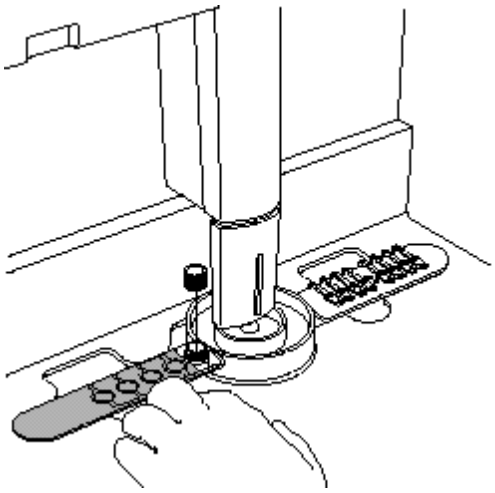



4. Remove the cap from the Enzyme vial.

5. Insert the vial into the carrier.

6. Place the DNA Prep carrier into the slot labeled **Reagent** to the left of the sample carrier slot.

7. Push the DNA Prep carrier down firmly until it snaps into place.

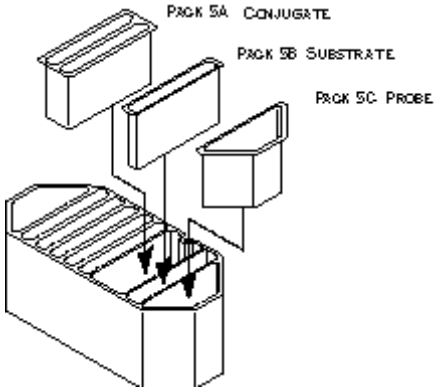


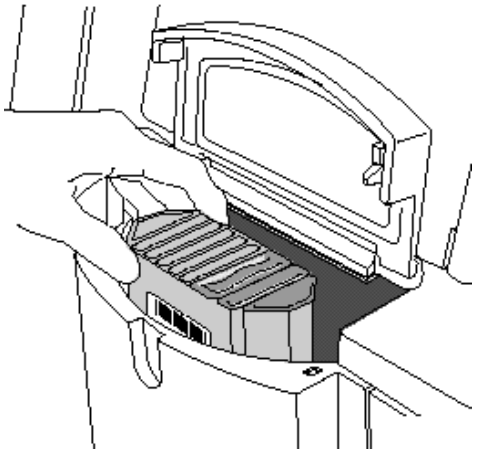
## 4. Load the MP Base and Carousel

1. Unpack the disposables.

2. Remove the MP base (Pack 5) from the incubator and the Conjugate (Pack 5A), Substrate (Pack 5B), and Probe (Pack 5C) from the refrigerator.

3. Remove each insert from its pouch. Tap the powdered reagent packs gently to bring all powder to the bottom of the packs. Place reagent packs in the MP base and load the base in the carousel.



*CAUTION! Push each insert firmly into place. If part of the insert extends above the top of the base, it could catch on the bottom of the deck and cause a system error. You could lose one or more batches as a result. Each insert is keyed by shape and cannot be inserted incorrectly.*

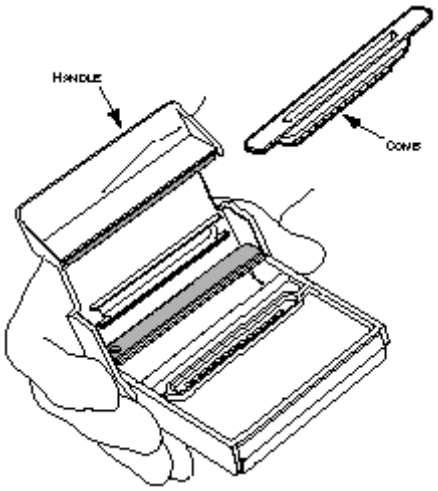
## 5. Load the Gel Cassette

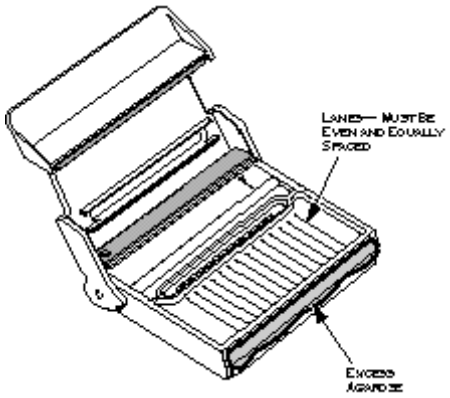
1. Remove the gel cassette from its package.

2. Grasp one end of the rubber comb and gently pull the comb from the cassette.

3. Unfold the handle of the cassette towards you until the handle snaps into place.

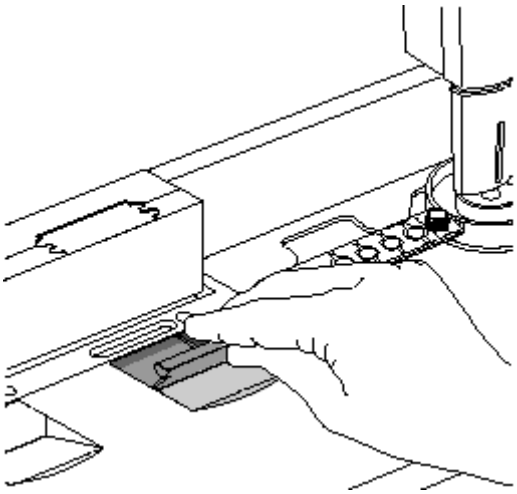
4. Check the front edge of the gel cassette and the lanes of the gel.




***Warning!*** *If the cassette shows a build up of excess gel on the front edge, or if you notice any shrinkage of the gel away from the cassette or bubbles, record the lot number and call Customer Support. Use a new cassette for this run.*

5. Insert the gel cassette into the slot labeled **Gel Bay.** The RiboPrinter® system will prevent the insertion of the cassette into the incorrect slot by blocking one slot with the LDD Pipette.

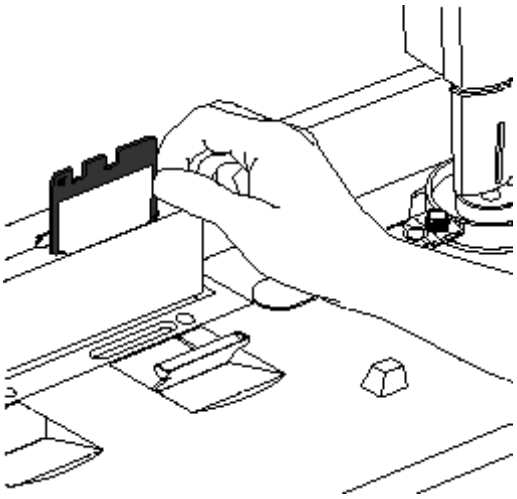


6. Press the cassette forward firmly until it snaps into place.

**6. Load the Membrane**

1. Grasp the membrane and carefully drop it into the front slot and flip the metal bracket against the back of the membrane.

*CAUTION! You can insert the membrane backwards. This will cause an alarm that prevents the sample from being processed until the error is corrected. Always make certain that the two large slots are on top and that the square hole on the side faces your left as you insert the membrane.*



**7. Close all doors and the instrument will begin sample processing.**

**8. Load the Next Batch**

The RiboPrinter® microbial characterization system lets you load up to four VCA batches in an eight hour period. Other batches may take longer to process.
The chart above shows the approximate loading times for each batch in a work shift using only the VCA protocol.

1. You can now use the **Create Batch** option to set up a new pending batch.

2. When you complete the information window and click on the **Start Normal Batch** option, the window displays a message telling you when you can load the next batch.

**Batch Report**

After image processing is completed, the system automatically runs a series of analysis functions and generates a Batch Information Report. This task does not require any action on the part of the operator. Reports are automatically saved to the hard disk of the computer and sent to the printer.